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# EUROPEAN MEDICAL JOURNAL

ISSN 2397-6764 Vol 1.2 • April 2016 • emjreviews.com

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# EUROPEAN MEDICAL JOURNAL



# Editorial Board

# European Medical Journal

**EUROPEAN MEDICAL JOURNAL** 1.2 | APRIL 2016 Published by the European Medical Group





The MedBIC, Anglia Ruskin University, Chelmsford, CM1 1SQ

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# Welcome

Hello and welcome to the second edition of the *European Medical Journal*, in which we present findings and insights discovered within the halls of hospitals, research facilities, and universities around the world.

The *European Medical Journal* series represents a range of therapeutic areas across the breadth of medicine; a promotion of the multidisciplinary approach to practice and the dispersion of information in the form of feature articles, peer-reviewed articles, and symposia.

Within this edition's exploration through the body, there is a fascinating respiratory-centric article by Minov et al., which discusses a potential link between chronic obstructive pulmonary disease and exposure to fumes in non-smoking welding workers. We also move into the world of neurology with a review by Gaulton and Gaulton that discusses the multifactorial impacts of emerging viral infections on neurological disease, using the West Nile, chikungunya, and Zika viruses to illustrate.

In our editor's pick, Ponce et al. analyse advances in the technical aspects of peritoneal dialysis, as well as the advantages and limitations of this treatment for acute and chronic kidney disease; this will be a vital piece of reading for anyone involved in the field of nephrology. Another highlight from this edition comes in the form of a comprehensive review of alcoholic liver disease, courtesy of Pal and Ray. These are just a few examples of the stimulating research you have to look forward to in this edition of the *European Medical Journal*; there are plenty of other peer-reviewed articles contained within, bridging topics such as interventional cardiology, hepatology, urology, and more.

This journal is also adorned by a series of feature articles, from a discerning 'How I Treat' piece focussing on lung cancer, to a consideration of how and why big data should be used in the advancement of cardiovascular research, and indeed medicine.

It is our sincere hope that this journal proves both useful and inspiring to you in your daily practice and research. We also hope that you, our readers, continue with us on our journey as we travel through hi-tech and up-to-date research across the realms of medicine. As always, our thanks go to our esteemed Editorial Board for their ongoing support, and to you the readers, as you are the reason we strive to produce such a high-quality publication.

Spencer Gore

*Director, European Medical Journal*

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# Foreword

**Prof Norbert Lameire**

*Emeritus Professor of Medicine and Nephrology, Medical Faculty of Ghent University, Ghent, Belgium.*

Dear colleagues,

I would like to welcome you all to this edition of the *European Medical Journal*, which covers an array of current topics in medicine.

Included within this edition are several feature articles which cover some of the latest innovations in medicine. For example, contained within is a discussion on the treatment of lung cancer and the changes that are happening in the care of small-cell and non-small-cell cancers, and an article which analyses how big data can help to improve treatments in cardiology and its relevance to the development of new research methods.

> "Another fascinating topic explored in this edition is about the role of manual thrombectomy for ST-elevation of myocardial infarction by Hoole and Flore."

This issue's collection of articles comprises key research on a range of topics, with a look at the use of acute peritoneal dialysis in acute and chronic end-stage kidney disease from Ponce et al., and an interesting overview of the applications of modern tools such as the sequencing of RNA in nephrology by Eikrem, which will also address its use in other fields. Another fascinating topic explored in this edition is about the role of manual thrombectomy for ST-elevation of myocardial infarction by Hoole and Flore. Here, the authors observe the development from early, small trials to later, large-scale multicentre trials, and the resulting changes in clinical practice. Additionally, Abdulla et al. discuss the ongoing search for a suitable therapy for non-alcoholic fatty liver disease, which is the most common cause of chronic liver disease in many developed countries.

There are many other articles, covering vital topics such as paraganglioma of the thyroid gland, the neurological effects of the Zika virus, and chronic obstructive pulmonary disease in welding workers, to name a few.

As usual, all papers were externally peer-reviewed, guaranteeing accurate content.

The editorial board of the journal wishes you pleasant reading and looks forward to meeting some of the readers personally at upcoming European congresses.

Kind regards,

**Norbert Lameire**

Emeritus Professor of Medicine and Nephrology, Medical Faculty of Ghent University, Ghent, Belgium; Past Chairman of the European Kidney Health Alliance.

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## INTRODUCTION

Lung cancer is the most common cancer and the leading cause of cancer-related deaths worldwide, accounting for about 1.8 million new diagnoses with 1.6 million deaths every year.[1] Lung cancer can be subdivided into two main groups: non-small cell lung cancer (NSCLC), accounting for about 85% of all new lung cancer diagnoses; and small-cell lung cancer (SCLC), which represents approximately 12–15% of all diagnoses. Considering that we are in the setting of a fairly complex clinical entity, the currently available guidelines are extremely helpful in providing evidence-based guidance to physicians in their daily practice. In Europe, the main and most widely used evidence-based guidelines available are those produced by the European Society of Medical Oncology (ESMO). These are regularly updated whenever evidence supporting the implementation of new diagnostic tools, technologies, or techniques become available.[2]

## SMALL-CELL LUNG CANCER

SCLC, strictly related to cigarette smoking, is characterised by a rapid doubling time, high growth fraction, and the early development of widespread metastases with a very poor prognosis. The incidence of SCLC has been falling over recent decades, likely due to the reduction in cigarette smoking rates and the routine addition of filters to cigarettes.[3] Staging of SCLC is traditionally based on the Veterans Administration Lung Study Group system, which classifies patients as having either limited-stage (LD) (disease which is limited to one hemi-thorax, with hilar and mediastinal nodes that can be encompassed within one tolerable radiotherapy portal), or extensive-stage (ED) (when disease has progressed beyond any-type limited stage) disease.[4] However, the International Association for the Study of Lung Cancer recommends applying the tumour, node, metastasis system staging to patients with LD-SCLC because it provides additional prognostic value allowing for better separation of stage-specific LD-SCLC survival curves compared with previous staging systems.[5]

### Limited Disease

In LD-SCLC, median overall survival (OS) and 2-year survival rates were 15–20 months and 20–40%, respectively, with 20–25% of patients surviving 5 years.[6] The standard of care for patients with LD-SCLC is concurrent chemo-radiotherapy with early thoracic radiotherapy (with chemotherapy cycle 1 or 2). The best OS in fit patients was demonstrated with twice-daily 1.5 Gy in 30 fractions given concurrently with 4 cycles of chemotherapy. In patients who are not fit enough for twice-daily radiotherapy or are unwilling to accept increased toxic effects, treatment with a once-daily (1.8 Gy, 25 fractions) radiotherapy schedule with 4–6 cycles of concurrent cisplatin plus etoposide is recommended. This intensive approach appears to be superior to sequential chemo-radiotherapy and yields higher OS. All patients without disease progression after treatment and reasonably good performance status (PS) should receive prophylactic cranial irradiation (PCI).[7]

### Extensive Disease

Despite a high objective response rate (ORR), close to 70%, outcomes of ED-SCLC patients remain poor with a median progression-free survival of only 5 months and a median OS of around 10 months. Platinum plus etoposide represents the standard treatment for patients affected by ED-SCLC and radiotherapy plays a local palliative role.[7] According to the results of an individual patient data (IPD) meta-analysis, cisplatin can be substituted by carboplatin in patients with ED-SCLC. Due to the limited number of LD-SCLC patients included in this analysis, cisplatin plus etoposide is still recommended in this group.[8] PCI should be offered to patients in a reasonably good PS with any response to first-line treatment.[7]

Although SCLC generally shows an excellent response to initial chemotherapy, most patients

ultimately relapse, for which salvage chemotherapy is an option to consider. For resistant patients with early relapse (<60–90 days after completion of first-line chemotherapy), participation in a clinical trial or best supportive care is recommended. Topotecan, either intravenous or oral, is recommended for patients having sensitive relapse, with CAV (cyclophosphamide, doxorubicin, vincristine) regimen as an alternative option. Platinum-based re-challenge, usually platinum plus etoposide, should be a further approach in very sensitive-relapsed SCLC patients.[7]

## NON-SMALL-CELL LUNG CANCER

NSCLC includes squamous cell carcinoma and non-squamous histology (adenocarcinoma, and large cell carcinoma). Regrettably, the percentage of patients affected by early-stage NSCLC and suitable for radical treatment as curative intent is low. Unfortunately, most patients are diagnosed when NSCLC is metastatic and systemic therapy is the mainstay of management. However, the long-term OS of patients affected by NSCLC, both in early and advanced NSCLC stages, remains low.

### Early Stages

Surgery, if feasible, should be the standard of care for Stages I and II of NSCLC, though careful evaluation is required for Stage III. Adjuvant platinum-based chemotherapy should be considered for patients with resected Stage II or III NSCLC.[9,10] An IPD meta-analysis showed that a cumulative cisplatin dose of 300 mg/m$^2$ delivered in 4 cycles is related to the best outcomes.[11] Cisplatin plus vinorelbine is the most frequently studied regimen and provides a superior OS benefit than other regimens; however, it is burdened with significant toxicity.[12] Thus, the selection of patients to treat with adjuvant treatment is very important to optimise results. Comorbidity, time-from-surgery, type of surgery, and post-operative recovery need to be taken into account in this decision. Post-operative radiotherapy is not recommended in radically resected NSCLC, but may be an option in Stage III based on critical evaluation of loco-regional relapse risks.[10]

### Locally-Advanced Disease

Stage III NSCLC represents a heterogeneous group of patients in which cure rates and long-term prognosis differ significantly between the various sub-stages. Thus, this also contributes to the difficulty of interpreting results. In the presence of an intra-operative diagnosis of Stage IIIA-N2 disease, surgery should be followed by adjuvant chemotherapy. The addition of post-operative radiotherapy is not routinely recommended, but may be an option following individual risk assessment. Several options should be considered in patients with a pre-operative diagnosis of Stage IIIA-N2 disease: induction chemotherapy or chemo-radiotherapy, followed by surgery or concurrent definitive chemo-radiotherapy. An experienced multidisciplinary team is of paramount importance in any complex multi-modality treatment strategy decision. Concurrent chemo-radiotherapy is the treatment of choice in patients with unresectable stages IIIA and IIIB NSCLC. In the presence of conditions contraindicating concurrent chemo-radiotherapy, sequential approaches of induction chemotherapy followed by definitive radiotherapy represent a valid and effective alternative. Platinum-based regimens, particularly containing cisplatin, are the treatment of choice.[10]

## METASTATIC DISEASE

In the last 10 years, improvements in the knowledge of the biological mechanisms of lung cancer have been growing, and the awareness of target molecules has led to the development of corresponding drug inhibitors. This is leading to 'personalised medicine', meaning the possibility of treating specific lung cancers with a precise genetic alteration which represents the target for a specific drug to be used in that specific individual. To date, only approximately 20% of NSCLC patients have a therapeutic drug target in clinical practice: activating mutations of epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) translocations. Gefitinib, erlotinib, and afatinib are oral small molecule anti-EGFRs, strongly recommended for first-line therapy in patients with advanced NSCLC harbouring activating EGFR mutations.[13] Most patients do not present any therapeutic drug targets and the choice of first-line therapy is based on histology, comorbidity, PS, and age. In squamous NSCLC, platinum-based doublets containing taxanes, gemcitabine, and vinorelbine are considered the standard of care. In non-squamous histology two further and strongly recommended options are platinum plus pemetrexed or carboplatin plus paclitaxel plus bevacizumab (an anti-vascular growth factor receptor monoclonal antibody) regimens. In this histotype, patients who do not progress to 4 cycles of platinum-pemetrexed induction,

maintenance therapy with pemetrexed is the standard of care demonstrating an OS advantage. In elderly (age ≥70 years) and PS2 patients unsuitable for platinum-based chemotherapy, single-agent is the preferred option.[13]

According to the results of two IPD meta-analyses, cisplatin-based chemotherapy is slightly superior to carboplatin-based chemotherapy in terms of ORR and, in certain sub-groups OS is slightly superior,[14] and 4 planned cycles of first-line platinum-based chemotherapy are enough.[15]

In second-line therapy, any patient with NSCLC harbouring an ALK fusion should receive crizotinib. Second-line therapy options consist of pemetrexed for non-squamous histology, docetaxel, and erlotinib, regardless of EGFR mutational status. Erlotinib is also the only drug registered for third-line therapy, if not received previously.[13]

Recently, a Phase III randomised trial showed an impressive improvement in OS for nivolumab, a monoclonal antibody immunotherapeutic directed against the programmed death-1 receptor, when compared in second-line therapy versus docetaxel in the treatment of patients with squamous NSCLC (median OS, 9.2 versus 6.0 months, respectively).[16] Based on the results to date, nivolumab is registered for this approach.

## CONCLUSION

Lung cancer is considered a 'big killer' and represents a challenging field of clinical research as many open questions remain, mainly concerning the best therapeutic approach of the disease. The knowledge of new therapeutic biological targets, such as the proto-oncogene, receptor tyrosine kinase (ROS1) rearrangements, for which crizotinib has already shown marked antitumour activity,[17] is of paramount importance to define sub-groups of patients who could benefit from 'personalised medicine'. Immunotherapy is a new frontier for the management of cancers, including lung cancer, with very promising preliminary results. The role of all professionals involved in the management of lung cancer is to keep up to date and ready to provide the best care to patients.

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# BIG DATA AND CARDIOLOGY: TIME FOR MASS ANALYTICS?

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**Disclosure:** Dr Rahul Potluri is the Founder of the ACALM Study Unit which undertakes clinical epidemiology research utilising anonymous routinely available data. Dr Ignat Drozdov is an employee of Bering Limited. The views expressed in the publication are his own and do not necessarily reflect those of the company.

The dawn of the millennium coupled with developments in technology has led to the computerisation of information previously collected with paper and filed in large warehouses. If the first decade of this century brought about the era of data collection, the current decade belongs to big data analysis. Big data covering topics such as how we shop, spend our money, go on holiday, seek healthcare, and finally the range of medical conditions we have, are all recorded, analysed, and scrutinised to the minutest detail for information, profit, and general and scientific knowledge; we have all benefitted from this.

In the commercial world, multinational giants such as Tesco have shown the way by introducing reward schemes, collecting information about consumer spending patterns and ingeniously encouraging customers to spend more. In healthcare, hospital and primary care records, demographics, medical conditions, and comorbidity and mortality information have all been captured in the majority of the Western world, primarily for financial reasons, but to a lesser extent for audit and monitoring purposes. At the touch of a button, analyses can inform about the financial status of a department, hospital, region, or even a country. However, the healthcare sector has been slow to realise the true potential of this information. There are an exponential number of methods in which this data can be used to plan clinical, social, and auxiliary services, offer patient-tailored care, develop the arguments for health economics, and advance medical science.

As cardiologists, it is the enhancement of medical science and the opportunity to develop tailored patient services that interests us the most with big data. Large databases in the field of cardiology are not new; the Framingham study,[1] which followed generations of patients from Framingham, Massachusetts, USA, has led the way since the 1950s and a number of clinical risk models are based on this study.[2-4] Scandinavian countries have large registry data from the 1960s[5,6] which have led to numerous large-scale studies.[7] Work from Birmingham, UK, has led to vast improvements in the study of common conditions such as atrial fibrillation and development of clinically useful risk scores, such as $CHA_2DS_2$-VASc,[8] to determine the stroke risk of these patients and the use of anticoagulation in their treatment. The development of the National Institute for Cardiovascular Outcome Research (NICOR) and the British Cardiovascular Intervention Society (BCIS) datasets in the UK have led to vast improvements in our understanding of acute coronary syndrome and interventional cardiology.[9,10] In the USA, Medicare datasets have aided in large analyses related to cost-benefit ratios of cardiovascular procedures and related conditions.[11] These datasets have also informed us about the growth of revascularisation procedures such as percutaneous coronary intervention and coronary artery bypass grafting.[12]

Cardiology is an evidence-based speciality and clinical trials make up the backbone of clinical decision making and management.[13,14] However, in the face of increased scrutiny and regulation, the costs of these trials are rising steeply and

have already run into billions of dollars. It is not uncommon to require a 20-year period for the development of a new treatment from its inception before it becomes mainstream, and the drawbacks and risks are large and very expensive. Therefore, with increasing technology and knowledge, it is disappointing to see the lack of significant major developments in the treatment of routine cardiovascular conditions, particularly when looking at medical therapy. Less expensive, registry-based clinical trials are being implemented.[15] In other cases, trials are designed with less significant endpoints and shorter follow-up periods to lower the cost. Moreover, none of these strategies hide the fact that patients in clinical trials are highly selected and in the face of an ageing population in the Western world, are less representative of real-world populations.

All the large datasets we have discussed so far in this article are collected data and/or registries ranging from hundreds of patients to many hundreds of thousands of patients. So we already have big data; why bother with more? Well, what about all that routinely collected data in the healthcare sector? The population of the UK is in excess of 60 million; the Western world, where such routine information has been collected over the last decade, has a population of over a billion. The developing world, which is not far behind in terms of data collection (namely Southeast and East Asia), has a population numbering multiple billions. Such enormous numbers pale the existing cardiology datasets, provided such datasets can be utilised, developed, and applied. The ACALM (Algorithm for Co-morbidities, Associations, Length of stay, and Mortality) study unit has been developed with such a dataset in mind. This algorithm differs from existing datasets because it utilises completely anonymous, routinely available healthcare information and transforms the data into fully functional, cross-sectional, and/or longitudinal research databases with real-life outcomes. The ACALM study unit is only 2 years old but has datasets of millions of patients and has already undertaken a number of studies addressing poorly researched areas, such as the interplay between mental and physical health in cardiology and factors influencing outcomes such as mortality and length of hospital stay.[16-18]

This is just the beginning. Datasets such as this one have the potential to not only answer questions that cannot be answered, but to revolutionise the way research is performed. It is the norm to perform literature reviews and to develop and test hypotheses with a carefully planned study. What if the research question is not known until the data is analysed? What if the data suggests information which we could not think of as plausible with our existing knowledge?

Complex modelling and further algorithms adapted from the world of computing, mathematics, and statistics can be used to enhance our knowledge and generate hypotheses for further research. Recent advances in both algorithm development and hardware infrastructure have paved the way for rapid adaptation of artificial intelligence and machine learning in medicine. Indeed, large-scale datasets generated in routine clinical settings can now be mined using sophisticated algorithms to accurately predict the onset of septic shock,[19] optimise patient-specific anticoagulation regimes,[20] and assess the prospective-risk of myocardial infarction.[21] Machine learning offers an opportunity to identify concepts rather than correlations in clinical data, thus promising to become an invaluable tool for data-aided decision making.

As with anything, there are limitations ranging from the quality of data collection, to the practicality of resourcing and running such large datasets, and other logistical and bureaucratic factors. However, the significant cost advantages of utilising routinely collected data, the sheer size of the datasets, and the fact that such data cannot otherwise be collected weigh heavily in favour of such research. We believe big data analytics will delineate a paradigm shift in cardiovascular medicine.

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# REGORAFENIB IN ADVANCED AND REFRACTORY GASTROINTESTINAL CANCERS

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**Disclosure:** Medical writing assistance was funded by Bayer.
**Received:** 08.02.16 **Accepted:** 09.03.16
**Citation:** EMJ. 2016;1[2]:18-25.

## ABSTRACT

The American Society of Clinical Oncology's 2016 Gastrointestinal Cancer Symposium (ASCO-GI), held in San Francisco, California, USA, provided a forum for leading basic scientists and clinical cancer specialists to discuss cutting-edge research in the field of gastrointestinal (GI) oncology. The quest to improve outcomes and patient lives by targeting unmet clinical need, such as refractory illness, fuelled much of the research presented at the 2016 edition of ASCO-GI. The symposium saw the presentation of a number of studies on the current stage of clinical research on regorafenib, an oral tyrosine-kinase inhibitor approved for use in both refractory metastatic colorectal cancer and metastatic GI stromal tumours.

## INTRODUCTION

Regorafenib is a promiscuous multikinase inhibitor which blocks the activity of several protein kinases involved with angiogenesis (vascular endothelial growth factor [VEGF] receptors 1–3 and TIE2), oncogenesis (KIT, RET, RAF1, B-RAF, and B-RAF V600E), and the tumour microenvironment (platelet-derived growth factor receptor [PDGFR] and fibroblast growth factor receptors [FGFR]).[1] Trials of regorafenib for metastatic colorectal cancer (mCRC) progressed rapidly from Phase I to completion of the Phase III CORRECT trial and subsequent worldwide approval as a third-line therapy within 2 years.[2] The rapid recruitment of these studies is an illustration of the previously unmet need in this patient population. In addition to mCRC, regorafenib is approved in gastrointestinal stromal tumours (GIST)[3] and Phase III trials for advanced oesophago-gastric cancer (AOGC) are underway.[4]

## SAFETY AND EFFICACY OF REGORAFENIB IN METASTATIC COLORECTAL CANCER IN THE UNITED STATES

The open-label, single-arm, Phase IIIb CONSIGN study (NCT01538680) (N=2,872) was designed to provide patients with refractory mCRC access to regorafenib prior to market authorisation, and to further assess the drug's safety and efficacy. CONSIGN was conducted in 25 countries in patients ≥18 years of age, with good (≤1) Eastern Cooperative Oncology Group performance status (ECOG PS). Patients had experienced progression at or within 3 months of therapy with approved treatment options and received regorafenib 160 mg/day on a 3 week on, 1 week off cycle. The primary efficacy outcome was progression-free survival (PFS) assessed per investigator according to local standards.[5]

Dr Udit Verma, University of Texas Southwestern Medical Center, Dallas, Texas, USA, presented a retrospective analysis of the US patient cohort from the CONSIGN trial, conducted in order to assess the safety and efficacy of regorafenib in patients from the USA.[6]

All patients assigned to treatment from the USA (N=364) received treatment and were included in the safety analysis. Median patient age was 60 years, and the majority of patients were white (80%). Notably, 38% of patients had wild-type *KRAS* and 59% had a mutated *KRAS* gene, which is a higher proportion of mutation than typically seen in mCRC.[7] Dr Verma speculated that this reflected the rapid progression of patients with *KRAS* mutations to a refractory state due to reduced treatment options.[8] The population was

characterised by advanced disease, with 82% of patients having been diagnosed with metastases ≥18 months prior to enrolment.

In the CONSIGN trial, patients from the USA appeared to achieve a longer median treatment duration (2.3 months, range: 0.03–30) than in the CORRECT study.[2] The median number of cycles was three (range: 1–33). The range of both outcomes indicates that some patients stayed on treatment for >2.5 years. The majority of patients started ≥3 cycles (55%), 19% started ≥6, and 10% started ≥9 cycles of therapy. The mean dose (±SD), excluding interruptions, was 148 (±17) mg/day, and the mean percentage of the planned dose was 77% (±20).

Treatment modifications, including dose reductions and re-escalations, and treatment interruptions or delays, occurred in 86% of patients. Dose reductions were carried out in 45% of these patients and the median duration of reduced dosing was 12 days (range: 1–45). A treatment interruption or a delay, the majority of which were brief (median 4.5 days, range: 1–33), occurred in 82% of patients.

Treatment-emergent adverse events (TEAEs) of Grade ≥3 occurred in 81% of patients, and were considered to be drug-related in 53% of patients. TEAEs leading to treatment modification occurred in 73% of patients, leading to a dose reduction in 43% of patients and discontinuation in 24%. Drug-related TEAEs leading to treatment modification occurred in 59% of patients.

The most common drug-related TEAEs of Grade ≥3 were hand-foot skin reactions (HFSRs; 16%), hypertension (15%), and fatigue (11%). Treatment-emergent hepatic and haematological laboratory values of Grade ≥3, which occurred regardless of relation to study drug, included increased bilirubin (9%), aspartate aminotransferase (6%), and alanine aminotransferase (3%); anaemia (5%); thrombocytopenia (2%); and neutropenia (2%). Median PFS was 2.3 months (Table 1), which was similar to data from the CORRECT trial (1.9 months). The effect of *KRAS* mutation on regorafenib efficacy in mCRC was investigated; however, PFS in the wild-type (2.1 months) and *KRAS*-mutant (2.3 months) sub-groups was similar in the CONSIGN USA cohort (Table 1).

To summarise, the safety profile of regorafenib in the US cohort was in line with the entirety of the international CONSIGN study, and the results were also similar to the CORRECT trial. PFS was similar irrespective of *KRAS* status and in line with results from CORRECT. The study demonstrated that a sub-group of patients respond very well to regorafenib (>2.5 years on the study drug). Characterising these individuals may offer an avenue to further investigate the presence of predictive biomarkers for regorafenib efficacy in this complex cancer, which has multiple potential contributory oncogenes.[9]

## SAFETY AND EFFICACY OF REGORAFENIB IN JAPANESE PATIENTS WITH METASTATIC COLORECTAL CANCER IN CLINICAL PRACTICE

The Westernisation of diet and lifestyle in Japan is thought to be linked to an expected 10-fold increase in colorectal cancer incidence between 1975 and 2020.[10] Regorafenib was approved for unresectable mCRC in Japan based on the results of the CORRECT study, where *post hoc* analysis showed comparable efficacy in Japanese (CORRECT-J, n=100) and non-Japanese subpopulations (n=660), and a manageable adverse event (AE) profile.[2,11]

At ASCO-GI, Dr Yoshito Komatsu, Hokkaido University Hospital Cancer Center, Sapporo, Hokkaido, Japan, presented an interim analysis of a post-marketing surveillance (PMS) study on the efficacy and safety of regorafenib in Japanese patients with mCRC.[12] Patients with unresectable metastatic or recurrent CRC were treated with regorafenib 160 mg/day in a 3 week on, 1 week off cycle. Dose modifications, including reductions and interruptions, were applied at the discretion of the physician, depending on the severity of drug related AEs. Outcomes were prospectively monitored for 6 months post-initiation and 1-year survival data were also assessed.

Data from 796 of the 1,303 enrolled patients were included in the current analysis (March 2013–August 2015), with 787 patients included in the safety and efficacy data sets. The majority of the baseline characteristics in the PMS cohort were similar to the regorafenib-treated CORRECT-J cohort (N=67), except for baseline ECOG PS (≥2: 10% versus 0%, respectively) and prevalence of *KRAS* mutations (47% versus 58%, respectively). The majority of PMS patients (66%) started at the planned daily dose of regorafenib; starting dose was not affected by ECOG PS.

**Table 1: Median progression-free survival (PFS) ±95% confidence interval (CI) in the USA CONSIGN cohort, and *KRAS*-wild-type and *KRAS*-mutant USA sub-groups.**

| CONSIGN sub-group | Number of patients (N) | Median PFS (months) | 95% CI |
|---|---|---|---|
| USA cohort | 364 | 2.3 | 2.0–2.6 |
| USA *KRAS*-wild-type patients | 138 | 2.1 | 1.8–2.7 |
| USA *KRAS*-mutant patients | 215 | 2.3 | 2.1–2.7 |

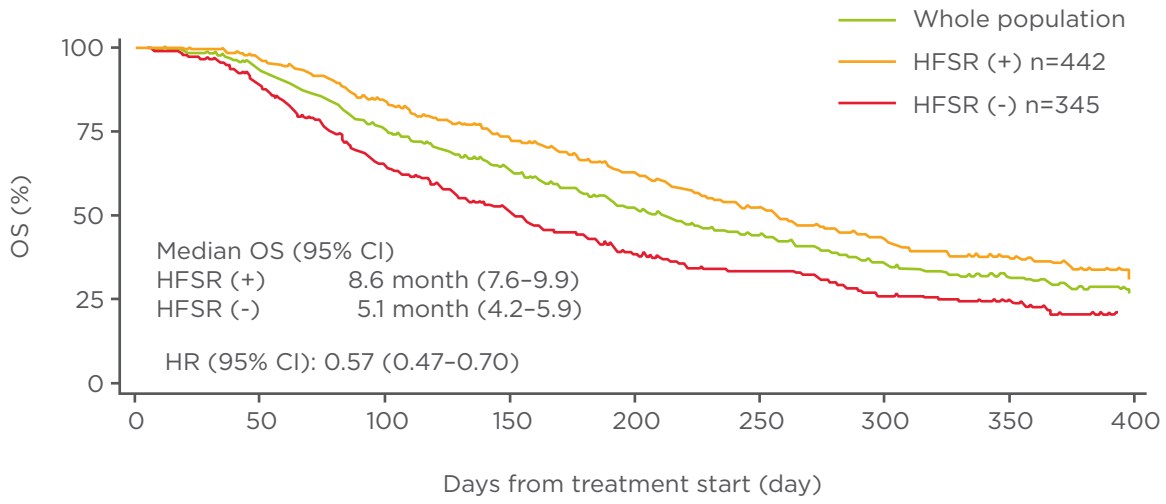


**Figure 1: Interim analysis of survival stratified by the presence of any grade of HFSR.**
HFSR: hand-foot skin reaction; OS: overall survival; HR: hazard ratio.

The majority of the 671 patients who had discontinued treatment at the time of analysis did so due to disease progression (58%). However, discontinuation due to drug-related AEs occurred more often in the PMS (37%) than in the CORRECT-J cohort (14%). Drug-related AEs of Grade ≥3 occurred in 51% of patients. The most common AEs were HFSR (18%), liver dysfunction (11%), hypertension (14%), thrombocytopenia (6%), fatigue (2%), and fever (1%). Although HFSR was the most common AE causing discontinuation, HFSR prophylaxis was not performed in all cases.

Common regorafenib-related AEs were most frequent in the first 3 weeks. The incidence of HFSR was highest in the second week (≈20%) and dropped to <5% from Week 5 onwards. The incidence of HFSR and liver dysfunction was lower in patients with a ≤120 mg/day initial dose than in patients starting on 160 mg/day.

Efficacy data were in line with the CORRECT-J population. Median (95% confidence interval [CI]) overall survival (OS) was 7.0 months (6.3–7.8; CORRECT-J, 6.6 months), and time-to-treatment failure was 2.1 months (1.9–2.2). Patients who had worse ECOG PS (≥2) had a shorter median OS (2.9 months [2.3–5.0]) than patients who had ECOG PS of 1 (5.9 months [5.2–6.8]) or 0 (9.9 months [8.4–11.6]).

Dr Komatsu indicated that the data from an exploratory analysis showing that median OS was longer in patients with HFSR than in those without HFSR was novel and in need of further investigation (Figure 1). Possible explanatory factors such as a higher dose of regorafenib have yet to be investigated. In addition, patients who developed HFSR during the first 4 weeks of treatment and who survived beyond Week 4 showed better median OS (8.3 months [7.0–9.5]) than those surviving at Week 4 who had not experienced HFSR (6.0 months [5.2–7.0]).

In conclusion, the interim results of the PMS suggest that the safety and efficacy profiles of regorafenib in Japanese patients in clinical practice is consistent with those from the CORRECT study. However, discontinuation due to regorafenib-related AEs occurred more often in the PMS than in the CORRECT-J cohort. The early onset of TEAEs suggest that frequent monitoring, particularly

in the early stages of treatment, may help physicians and patients manage drug-related AEs. In addition, dose modification and appropriate AE management including prophylaxis may help reduce discontinuation. The findings of the exploratory analysis suggesting a possible relationship between occurrence of HFSR and better OS warrants further investigation in the full analysis set and beyond.

## EVALUATION OF CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR BASED BIOMARKERS IN THE INTEGRATE TRIAL

INTEGRATE (ANZCTR12612000239864) was a Phase II trial of regorafenib versus placebo (2:1; N=152) in refractory AOGC with crossover to active treatment allowed for patients in the placebo arm after progression. This multicentre, international trial recruited patients from countries including Australia, New Zealand, Canada, and Korea. Regorafenib was highly effective in prolonging PFS (11 weeks) compared with placebo (4 weeks). Although regorafenib was effective across geographical regions, the effect on PFS was significantly greater in Korea.[13]

Dr Sonia Yip, Senior Translational Research Fellow, NHMRC Clinical Trials Centre, University of Sydney, Sydney, Australia, presented a translational biomarker study on data from the INTEGRATE trial which aimed to predict which patients would benefit most from regorafenib.[4] Patients' blood samples were collected (N=145) at three time points: baseline, and Day 1 of cycles two and four. Dr Yip presented analyses VEGF isoforms (VEGF-A, B, C, and D) and serum vascular endothelial growth factor receptors (sVEGFR-1, 2, and 3), as well as interleukin (IL)-8, at baseline. As in the majority of cancers, high VEGF and circulating sVEGFR expression, which indicate upregulation of angiogenesis pathways, are associated with poor prognosis in gastric cancer.[14-16]

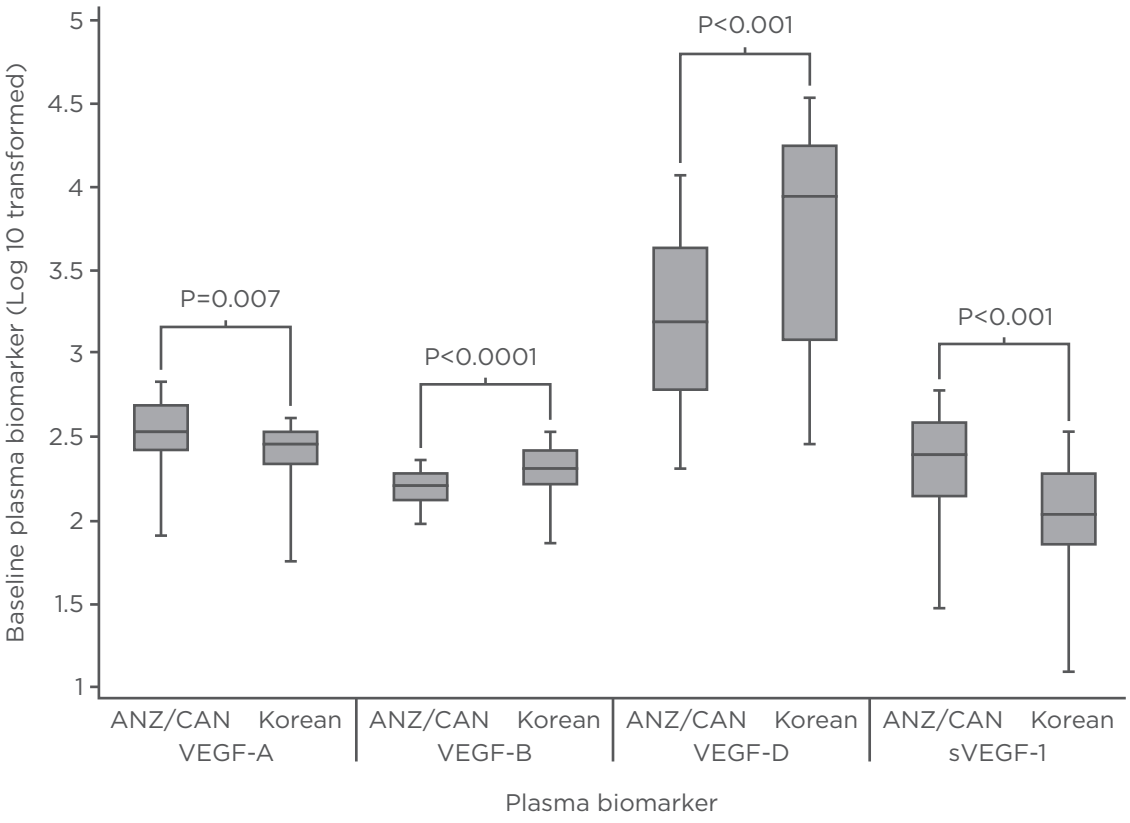


**Figure 2: Median levels of circulating biomarkers in patients from Australia, New Zealand, and Canada versus those from Korea.**

ANZ: Australia and New Zealand; CAN: Canada; VEGF: vascular endothelial growth factor; sVEGFR: serum vascular endothelial growth factor receptor.

All P values based on Wilcoxon test on median values. Boxes, median (±interquartile range). Whiskers, maximum and minimum.

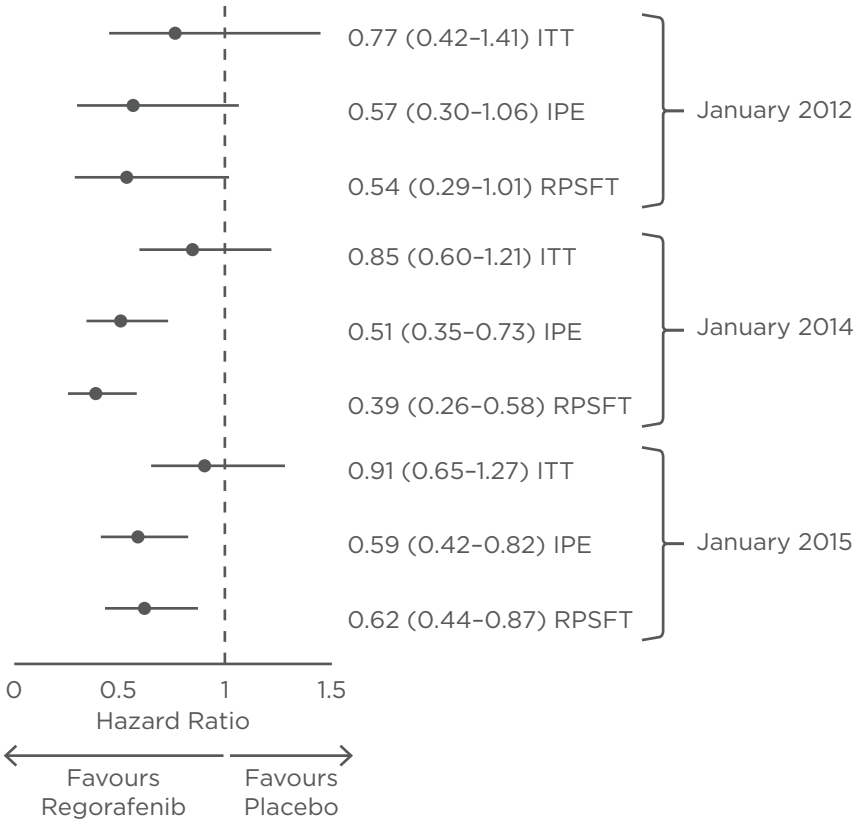


**Figure 3: Forest plot of hazard ratio for overall survival in unadjusted (intent-to-treat) and model populations at three time points.**

ITT: intent-to-treat, IPE: iterative parameter estimation; RPSFT: rank-preserving structural failure time.

Analysis of baseline markers revealed regional differences, with higher VEGF-A and sVEGFR-1 levels in patients from Australia, New Zealand, and Canada, compared with Korean patients (Figure 2). In contrast, VEGF-B and D isoforms were higher in Korean patients (Figure 2). The effect of region on the efficacy of regorafenib that was observed in the initial analysis of INTEGRATE was also maintained when evaluated in a multivariate analysis alongside baseline biomarkers. The physiological or treatment-related basis of regional differences in efficacy and VEGF-isoform levels remains to be elucidated.

Novel results were revealed in a correlation analysis of biomarkers with a very strong positive correlation existing between the plasma levels of VEGF-C and VEGF-A (r=0.88) and a strong correlation between IL-8 and VEGF-A, C, and D (r=0.57, 0.68, 0.66; respectively). A modest negative correlation was found between VEGF-D and sVEGFR-1 (r=-0.33).

Hazard ratios (HR [95% CI]) initially suggested a prognostic relationship between PFS and IL-8 (1.89 [1.01–3.56], p=0.047), VEGF-A (1.14 [1.01–1.30], p=0.037), and sVEGFR-1 (1.64 [1.01–2.66], p=0.045). However, the significance of these putative prognostic markers was lost when adjusting for age and neutrophil-to-lymphocyte ratio (NLR). A high NLR is a strong negative prognostic marker in gastric cancer and was found to be a strong prognostic marker for both poorer PFS (HR 1.56, p=0.01) and poorer OS (HR 1.83, p=0.001) in the INTEGRATE study.[17] Once again regional differences emerged, with lower NLRs observed in Korean patients (mean, 3.1 [2.3]) compared with patients from Australia, New Zealand, and Canada (mean, 5.4 [4.6], p<0.001). However, despite this indicator for better prognosis existing in Korean patients, who responded better to regorafenib, NLR was not found to be predictive of regorafenib efficacy.

In summary, despite novel study findings in terms of regional differences and correlations between VEGF and VEGFR isoforms, a predictive biomarker for the indication of regorafenib benefit remains elusive. Dr Yip and colleagues are continuing to analyse a broad base of biomarkers beyond the VEGF axis (IL-6, TIE-1, TIE-2, FGFR, PDFR, PDGFR, PAI-1, and PAI-2), other time points from within the

regorafenib treatment cycle will be investigated in addition to tissue biomarkers. Putative results will be used to inform the biomarker analysis of the Phase III INTEGRATE II study.[4]

## OVERALL SURVIVAL ANALYSIS: MODELLING CROSSOVER IMPACT IN ADVANCED GASTROINTESTINAL STROMAL TUMOURS

The management of GIST has radically improved over the last 16 years, driven by an understanding of the root cause of most cases of the disease, mutated tyrosine-kinase signalling.[18] A series of highly potent tyrosine kinase inhibitors (TKIs; imatinib, sunitinib, and regorafenib), are now approved by regulatory authorities worldwide for the treatment of unresectable and metastatic GIST.[19]

ASCO-GI saw data presented from an exploratory analysis on the Phase III GRID trial of regorafenib in advanced GIST, conducted by a team led by Prof George D. Demetri, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts, USA.[20] GRID (NCT01271712) was a randomised, multinational trial of regorafenib (160 mg/day, 3 weeks on, 1 week off) versus placebo (2:1; N=199). The primary endpoint was PFS with OS as a secondary endpoint, allowing the trial to be designed with a crossover to open-label regorafenib for patients initially randomised to placebo whose GIST progressed objectively. Regorafenib significantly prolonged PFS compared with placebo (median PFS, 4.8 months versus 0.9 months, HR 0.27 [0.19, 0.39], p<0.0001). However, there was no significant difference in OS at the time of primary-endpoint assessment due to low numbers of death in the initial analysis and, likely also to the confounding effect of placebo patients crossing to open-label regorafenib.[3]

The advantages of the PFS endpoint in allowing crossover to active therapy is recognised by the Food and Drug Administration (FDA).[21] Dr Demetri noted that this type of trial design was particularly advantageous to patients with advanced TKI-resistant GIST where progression commonly occurs in <1 month and a fatal outcome from uncontrolled disease is rapid.[3] In addition, crossover design may also address patient concerns that placebo-controlled trials withhold treatment from those in need. However, the use of a crossover design, particularly in the case of a highly-active study drug and rapidly-progressing disease, has the disadvantage of confounding OS as an endpoint due to swift mixing of the placebo group with patients who cross over to receive the active study drug.

The current exploratory analysis was conducted on long-term follow-up data from patients enrolled in GRID and tested two statistical methods designed to model the effect of patients continuing on placebo without crossover. Beyond assessing the effect of regorafenib on OS in advanced GIST, the study aimed to compare exploratory analyses that adjusted for the effect of crossover on OS using high-quality study data. Dr Demetri and colleagues compared two established randomisation-based statistical methods, rank-preserving structural failure time (RPSFT) and iterative parameter estimation (IPE).[22,23] Analyses were conducted at three different points of data collection (January 2012, January 2014, and June 2015), each of which included the full randomised patient population.

Characteristically for patients with advanced disease, participants had been heavily pre-treated. Close to half (43%) of patients had received >2 lines of therapy previously, indicating that they had been treated with experimental/investigational or off-label TKIs in addition to the standard imatinib and sunitinib, which all participants had received.

At analysis of the primary endpoint in the GRID study in 2012, 46 deaths (23% of total study population) had occurred. Mean (SD) time on regorafenib was 5.5 months (2.8) for the regorafenib arm and 3.5 months (2.1) for the placebo arm. The majority of placebo-treated patients (n=56, 85%) had crossed over to regorafenib immediately after progression. By the 2014 data analysis, 139 (70%) deaths had occurred, and mean time on regorafenib was 12.6 months (10.4) and 9.7 months (8.7) for the regorafenib and placebo arms, respectively. At this time point, all surviving patients were receiving regorafenib and 58 (88%) of the placebo arm had crossed over. At the final analysis time point, 162 (81%) deaths had occurred and mean time on regorafenib was 14.0 months (13.2) and 10.8 months (11.3) for the regorafenib and placebo arms, respectively.

Kaplan–Meier curves showed that survival probability in the unadjusted placebo arm was statistically similar to that of the regorafenib arm at all time points analysed, indicative of the effect of crossover to open-label regorafenib. However, in both models statistically adjusted for the impact of

crossover, survival probability diverged, showing a marked advantage for patients in the regorafenib arm over the modelled results of placebo treatment alone. Dr Demetri noted that the survival curves for the IPE and RPSFT were strikingly similar, a reassuring sign in terms of the statistical validity of the models.

HRs for the unadjusted intent-to-treat (ITT) population and the two models are illustrated in Figure 3. In 2012, the HR for the unadjusted ITT population indicated a 23% decrease in the risk of death in the regorafenib arm. The decreased risk fell to 15% and 9% for the 2014 and 2015 time points, respectively, further illustrating the effect of post-progression crossover treatment in the placebo arm. In contrast, the decreased risk of death in the IPE model was 43%, 49%, and 41%, at the 2012, 2014, and 2015 time points, respectively. Data from the RPSFT model showed a 46%, 61%, and 38% decrease in the risk of death at the three respective time points. The data from the IPE model appeared more stable, suggesting this may be the preferred model for future studies. Dr Demetri emphasised the benefit shown in the two models which was of a magnitude rarely reached within clinical trials in oncology. This was backed up by Kaplan–Meier analysis of the ITT population showing that approximately 12% of patients with advanced TKI-resistant GIST were still alive after 4 years on regorafenib.

In his conclusion, Dr Demetri discussed two potentially exciting implications from the current study, beyond the positive treatment effect. Firstly, the use of these statistical models on data from a high-quality clinical trial increases the understanding of the effect of the increasingly utilised and ethically sound crossover clinical-trial design on the OS endpoint. Secondly, the relative preponderance of long responders to regorafenib treatment combined with the comparatively simple tumour biology found in GIST may provide a small but powerful pool of participants for future predictive-biomarker studies on regorafenib.

## CONCLUSION

Regorafenib has shown efficacy in different types of advanced and metastatic gastrointestinal (GI) cancers in randomised trials. Prospective studies have now replicated both efficacy and safety data from these trials in the clinical setting. Identifying the patient population who will benefit most from regorafenib treatment remains challenging and research continues to identify predictive biomarkers, towards a rational placement of regorafenib in the optimal care of patients with GI cancers. Studies on the growing population of patients who show long-term benefit from regorafenib treatment may offer an avenue of research. Finally, the use of the modelling in crossover studies will be of use across the field of oncology and regulatory science to estimate efficacy in terms of OS in refractory disease states where a lack of crossover may not be ethically tenable.

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# EDITOR'S PICK

In this edition's Editor's Pick, Ponce et al. tackle the various complexities of urgent-start peritoneal dialysis in both acute and chronic kidney failure in light of the current use of haemodialysis, extending their study beyond the controversies and limitations of its use. This in-depth review focusses on the available evidence and guidelines for unplanned chronic dialysis in acute kidney injury, and combines the available evidence to provide a suitable guide to safely prescribing, delivering, and monitoring high volume peritoneal dialysis in patients.

# URGENT START PERITONEAL DIALYSIS: A VIABLE OPTION FOR ACUTE AND CHRONIC KIDNEY FAILURE

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**Disclosure:** Daniela Ponce has received a research grant from the Baxter Healthcare Corporation. Dayana Bittencourt Dias and Andre Luis Balbi have declared no conflicts of interest.
**Received:** 21.10.15 **Accepted:** 12.02.16
**Citation:** EMJ. 2016;1[2]:26-33.

## ABSTRACT

Peritoneal dialysis (PD) may be a feasible, safe, and complementary alternative to haemodialysis, not only in the chronic setting, but also in the acute. Recently, interest in using PD to manage acute kidney injury (AKI) patients has been increasing. Some Brazilian studies have shown that, with careful thought and planning, critically ill patients can be successfully treated with PD. To overcome some of the classic limitations of PD use in AKI, such as a high chance of infectious and mechanical complications, and no control of urea, potassium, and bicarbonate levels, the use of cycles, flexible catheters, and a high volume of dialysis fluid has been proposed. This knowledge can be used in the case of an unplanned start on chronic PD and may be a tool to increase the PD penetration rate among incident patients starting chronic dialysis therapy. PD should be offered in an unbiased way to all patients starting unplanned dialysis, and without contraindications to PD. In the following manuscript, advances in technical aspects and the advantages and limitations of PD will be discussed, and recent literature on clinical experience with PD use in the acute and unplanned setting will be reviewed.

Keywords: Peritoneal dialysis (PD), acute kidney injury (AKI), unplanned start.

## THE ROLE OF PERITONEAL DIALYSIS FOR ACUTE KIDNEY INJURY PATIENTS

In the 1970s, acute peritoneal dialysis (PD) was widely accepted for the treatment of acute kidney injury (AKI), but this practice has declined in favour of haemodialysis (HD).[1-4] PD is frequently used in developing countries because of its lower cost and minimal infrastructural requirements.[4-7] However, in developing countries, the infrastructure for quality research is often lacking, meaning that there has been limited evidence on standardised treatment regimens such as indications, dosing and technical failure, and mortality.

### Technical Aspects and Controversies

Use of PD in AKI is enhanced by placement of a Tenckhoff catheter by a nephrologist, which can be safely accomplished at the bedside.[8] PD offers several advantages over HD, such as technical

simplicity and a lower risk of bleeding. The gradual and continuous nature of PD ensures that disequilibrium syndrome is prevented and that cardiovascular stress is minimal, which reduces the risk of renal ischaemia and fluid-electrolyte imbalance.[1-8]

Besides the classical indications (volume overload, electrolyte disorders, uraemic symptoms, or acid-base disturbances), PD can also be used to maintain volemic control in patients with congestive heart failure (functional Class IV), and control hyper and hypothermia.[6-10] In the setting of natural disasters, when several victims will develop AKI and damage to infrastructure makes access to power, clean water, and facilities for water treatment unavailable, PD is an important and life-saving renal replacement therapy (RRT) modality.[8-12]

It is also true that PD is not the most efficient therapy: clearance per exchange can decrease if a shorter dwell time is applied, a lower efficiency can be observed in large-sized and severely hypercatabolic patients, fluid removal can be unpredictable, there is a risk of infection, and there are possible issues with mechanical ventilation.[5-15] PD is relatively contraindicated in patients with recent abdominal surgery, abdominal hernia, adynamic ileum, intra-abdominal adhesions, peritoneal fibrosis, or peritonitis. Table 1 shows the advantages and disadvantages of PD.

Since volume and solute removal is slow and unpredictable, PD is not as efficient as extracorporeal blood purification techniques for the treatment of emergencies such as acute pulmonary oedema or life-threatening hyperkalaemia.[11-17] Another possible limitation of PD in AKI is that associated protein losses may aggravate malnutrition. Protein losses as high as 48 g/day have been reported, but some reports document maintenance of serum albumin levels.[18-21] Protein supplementation, either enteral or parenteral (1.5 g/kg/day) is recommended for AKI patients on PD.[22]

The high glucose concentrations in peritoneal dialysate may cause hyperglycaemia, even in non-diabetic patients. This is easily correctable through intravenous or intraperitoneal administration of insulin.[21] Peritonitis occurring in patients with AKI using PD as a modality of RRT can lead to very poor outcomes, and older studies report a frequency as high as 40%.[2,3,6] With better catheter implantation techniques and automated methods, the incidence of peritonitis has been reduced and the risk of infection in PD is similar to other forms of extracorporeal blood purification for AKI.[2,3]

Previous studies have reported that PD can increase intra-abdominal pressure (IAP), which leads to impaired diaphragm mobilisation, and decreased pulmonary compliance and ventilation, which may cause or worsen respiratory failure.[22,23] However, PD is seldom the cause of ventilation impairment in patients without pulmonary disease.[24] Results from our group suggest increases in the pulmonary compliance without changes in IAP in AKI patients treated with PD.[25]

## Evidences and Guidelines

Recently, interest in using PD to manage patients with AKI has been increasing. The first question that must be asked is whether PD can provide adequate clearance in the treatment of AKI patients. Our study group, from the Botucatu School of Medicine, Brazil, demonstrated that, with careful thought and planning, critically ill AKI patients can be successfully treated with PD.[2,11,26,27] To overcome some of the classic limitations of PD use in AKI (such as a low rate of ultrafiltration [UF], high chance of infectious and mechanical complications, and no metabolic control) we proposed the use of cyclers, flexible catheters, continuous therapy (24 hours), and high volumes (HV) of dialysis fluid.

We assessed the efficacy of HVPD in a prospective study of 30 consecutive AKI patients.[11] PD was performed using a Tenckhoff catheter, 2 L exchanges, and 35–50 minute dwell times. The prescribed Kt/V value was 0.65 per session, the duration of each session was 24 hours, and a total dialysate volume of 36–44 L/day was used. HVPD was effective in the correction of blood urea nitrogen (BUN), creatinine, bicarbonate, and fluid overload. Weekly Kt/V was 3.8±0.6 and the mortality was 57%. Five years later, we performed another prospective study on 204 AKI patients treated with HVPD (prescribed Kt/V=0.60/session).[27] Sepsis was the main cause of AKI (54.7%) followed by heart failure (24.7%). BUN and creatinine levels stabilised after four sessions to approximately 50 mg/dL and 4 mg/dL, respectively. Weekly-delivered Kt/V was 3.5±0.68 and the mortality rate was 57.3%. Older age and sepsis were identified as risk factors for death. Persistence of urine output, increases of 1 g/day in nitrogen balance (NB), and achieving 500 mL/day

in UF after three sessions were identified as favourable prognostic factors. We concluded that HVPD is effective in selected patients. However, if after three sessions, UF is low or NB is negative, substitution or addition of HD should be considered. There were mechanical complications in 7.3% of AKI patients treated with HVPD and 12% of patients had infectious complications (peritonitis). Change of the dialysis method occurred in 13.3% of patients because of refractory peritonitis or mechanical complications (leakage or UF failure).

Dialysis dose adequacy in AKI is a controversial subject and there are very limited data on the effect of PD dose on AKI. Solute clearance in PD is limited by dialysate flow, membrane permeability, and surface area in contact with dialysate. Exchanges of 2 L lasting approximately 1 hour can achieve a saturation of the spent dialysate in the range of 50%. This means that, over 24 hours, a daily Kt/V of 0.5 can be achieved in a patient with a body weight between 60–65 kg.[2,9-11]

We performed a trial of 61 septic AKI patients randomised to receive higher (n=31) or lower (n=30) intensity PD therapy (prescribed Kt/V of 0.8/session versus 0.5/session). The two groups had similar mortality after 30 days (55% versus 53%, p=0.83). We concluded that increasing the intensity of continuous HVPD therapy does not reduce mortality and does not improve control of urea, potassium, and bicarbonate levels.[28]

According to the International Society for Peritoneal Dialysis (ISPD) guidelines: PD for AKI recommendations, where resources permit, targeting a weekly Kt/V urea of 3.5 provides outcomes comparable to that of daily HD; targeting higher doses does not improve outcomes. This dose may not be necessary for many AKI patients and targeting a weekly Kt/V of 2.1 may be acceptable.[29]

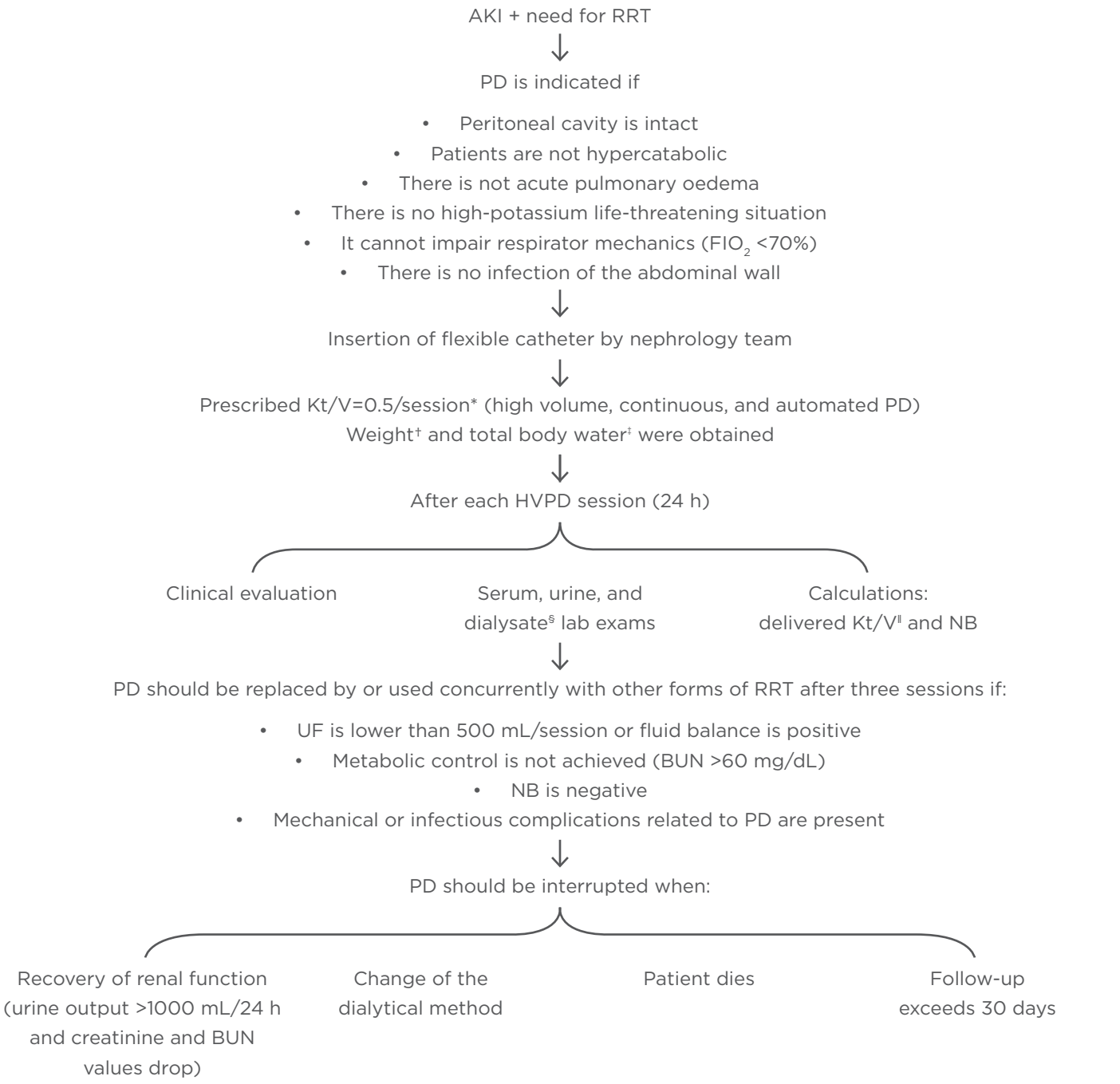
The second question to consider is whether PD is comparable to other dialysis methods in AKI patients. The answer to that question is neither simple nor currently complete. The various modalities present advantages and disadvantages under specific circumstances and these therapies should therefore be considered more as a continuum than as a series of modalities to be compared.[30,31] Few studies have compared PD with other dialysis methods in AKI patients, and reports conflict with regard to efficacy and cost. Phu et al.[16] compared intermittent PD with continuous RRT, and demonstrated a worse outcome in patients treated with PD. Such reports should not be underestimated, although specific factors (such as the use of rigid catheters, manual exchanges, a too-short dwell time [15 minutes], and no dialysis dose quantification)[4,6] might be involved.

A randomised study performed by our group in 120 AKI patients compared HVPD versus daily intermittent HD.[26] Baseline characteristics were similar in both groups, which included older patients (mean age >60 years), patients with a high APACHE II score, and patients using vasoactive drugs (>60%). Both RRT modalities achieved metabolic and acid-base control. Mortality did not differ significantly between the two groups (58% versus 53%). Renal recovery was similar for both modalities, but HVPD was associated with a significantly shorter time to recovery (7.2±2.6 versus 10.6±4.7 days).

**Table 1: Advantages and disadvantages of peritoneal dialysis in acute kidney injury (AKI).**

| Advantages | Disadvantages |
|---|---|
| • Technically simple<br>• No need for expensive equipment<br>• Avoids vascular access<br>• Ensures minimal blood loss<br>• Biocompatible<br>• Useful in all types of AKI<br>• Should enhance renal recovery<br>• Provides continuous RRT<br>• Beneficial in select patient populations (children, heart failure, cirrhosis, bleeding diathesis) | • Requires intact peritoneal cavity with adequate membrane function<br>• May not be adequate for severe acute pulmonary oedema or life-threatening hyperkalaemia<br>• Infection (peritonitis) can occur<br>• Ultrafiltration and clearance cannot be exactly predicted<br>• Can cause protein losses<br>• Can cause hyperglycaemia and hypernatraemia<br>• Can impair respiratory mechanics<br>• Lactate buffer |

RRT: renal replacement therapy.




*Prescribed Kt/V
K = volume of dialysis solution prescribed in 24 hours (mL) × 0.60 (considering the D/P relationship for dwel time between 30 and 60 min); t = 1 (day) and V = patient urea distribution volume (mL).
The number of exchanges was obtained by dividing the K value by 2 L (volume infused in the peritoneal cavity per exchange).

†digital scales or two variable formula[42] ‡Watson formula[43]

§Every day all effluent should be collected to calculate delivered Kt/V and NB. Every 3 days a cell count and cell culture should be performed to diagnose peritonitis.

‖Delivered Kt/V
Kt/V = mean dialysate urea nitrogen (mg/dL) / mean serum urea nitrogen pre and post dialysis (mg/dL) × drained 24 h volume (mL) / patient urea distribution volume (mL).

**Figure 1: Flowchart of the practical aspects of prescribing, delivering, and monitoring the HVPD in AKI patients.**

AKI: acute kidney injury; RRT: renal replacement therapy; PD: peritoneal dialysis; HVPD: high volume peritoneal dialysis; NB: nitrogen balance; UF: ultrafiltrate; BUN: blood urea nitrogen.

George et al.[30] performed a randomised study to compare continuous venovenous haemodiafiltration (CVVHDF) and PD in critically ill patients. No difference was observed in correction of metabolic parameters and fluid overload. Urea and creatinine clearances were higher and fluid correction was faster with CVVHDF. The mortality rates in the two study groups were similar. Unfortunately, the procedures were performed at different technological levels to the detriment of PD, in which rigid catheters, locally available PD fluids, and manual exchanges were used.

In another prospective study, we compared the effect of HVPD against prolonged HD (PHD) on AKI patients' outcome.[32] The PHD and HVPD groups were similar in gender, severity, and aetiology of AKI. There was a trend toward statistical difference regarding the presence of sepsis (62.3% in PHD group versus 44.9% in HVPD group, p=0.054). Delivered Kt/V and UF were higher in PHD group and there was no difference between the two groups in mortality and recovery of kidney function, or need for chronic dialysis.

A systematic review published by Chion et al.[33] concluded that there is currently no evidence to suggest significant differences in mortality between PD and extracorporeal blood purification in AKI, and that there is a need for high-quality evidence in this important area. Recently, a Brazilian group published the largest cohort study providing patient characteristics, clinical practice, patterns, and their relationship to outcomes in a developing country.[34] Its objective was to describe the main determinants of patient and technique survival, including trends over time of PD treatment in AKI patients.

For comparison purposes, patients were divided into two groups according to the year of treatment: 2004–2008 and 2009–2014. A total of 301 patients were included, though 51 were transferred to HD (16.9%) during the study period. The main cause of technique failure (TF) was mechanical complication (47%) followed by peritonitis (41.2%). There was a change in TF during the study period; patients treated during 2009–2014 had a relative risk (RR) reduction of 0.86 (95% CI, 0.77–0.96) compared with patients treated between 2004 and 2008, and three independent risk factors were identified: period of treatment at 2009 and 2014, sepsis, and age >65 years.

During the study there were 180 deaths (59.8%). Death was the leading cause of dropout (77.9% of all cases), mainly due to sepsis (58.3%), followed by cardiovascular disease (36.1%). The overall patient survival rate was 41% at 30 days and patient survival improved along study periods. Compared with patients treated from 2004–2008, patients treated at 2009–2014 had a RR reduction of 0.87 (95% CI, 0.79–0.98). The independent risk factors for mortality were sepsis, age >70 years, Acute Tubular Necrosis Individual Severity Score (ATN-ISS) >0.65, and positive fluid balance. In conclusion, we observed an improvement in patient survival and TF between the two time periods, even after correction for several confounders and using a competing risk approach. We have prepared a flowchart of the practical aspects of prescribing, delivering, and monitoring the HVPD in AKI patients (Figure 1).

This review clearly shows that PD is a simple, safe, and efficient way to correct metabolic, electrolytic, acid-base, and volume disturbances generated by AKI; it can be used as a RRT modality to treat AKI, either in or out of the intensive care unit setting. We have recently observed an improvement in patient and technique survival over the years even after correction for several confounders.

## THE ROLE OF PERITONEAL DIALYSIS FOR UNPLANNED INITIATION OF CHRONIC DIALYSIS

Although historically PD was widely used in nephrology, for reasons that are unclear it has been underutilised in recent years. Possible reasons for this include the 'perception' that it is inferior to HD, which is associated with greater technology; the infectious, mechanical, and metabolic complications associated with PD; the higher financial reimbursement with HD use; and difficulties with catheter peritoneal insertion.[35,36]

In 2007, there were 368,000 prevalent patients on RRT in the USA, 92.8% of whom were on HD.[37] Data from 2013 has shown that in Brazil, 90.6% of chronic patients underwent HD and only 9.4% were treated by PD.[38] Several studies have compared the differences between the two types of dialysis, PD versus HD, in incident patients on RRT. There is no evidence of the superiority of one method over the other in regard to general mortality within the first 2 years of therapy.[39-44] Some studies have demonstrated better results with PD in young

patient groups with no comorbidities, while other studies have shown lower mortality after 2 years of dialysis in elderly patients with comorbidities treated by HD.[40-42]

Some authors have recently highlighted the impact that the use of vascular access has in the mortality of incident patients in HD.[40,41] These studies found that central venous catheter (CVC) use is associated with reduced survival, especially in the first 90 days of RRT. Furthermore, there is a greater risk of bacteraemia, sepsis, and hospitalisation in patients using CVC when compared with patients using arteriovenous fistulas or PD.[45-47]

In this scenario, PD appears as an option in unplanned initiation of chronic dialysis. Advantages of PD include the lack of CVC use, thereby preserving vascular access and residual renal function, which can reduce the morbidity and mortality of these patients.[35,46,47] Most patients with end-stage chronic kidney disease (CKD) start unplanned RRT.[48-50] Ivarsen et al.[45] retrospectively reviewed the Danish Registration Nephrology from 2008–2011 and found that 50% of incident patients on RRT started the treatment in an unplanned manner. In Brazil, approximately 60% of incident patients on RRT have no definitive access and need to be treated through CVC. In the dialysis unit of the University Hospital of the Botucatu Medical School, the reality is worse than in the rest of the world: more than 90% of the incident patients start unplanned dialysis and 60% of prevalent patients have no functioning vascular access and are treated through CVC.[49,50] Unplanned dialysis may be defined as the start of HD without functioning definitive vascular access, i.e. using CVC, or as the start of PD <7 days after its implantation.[45-50] This situation is common even for patients who have attended a previous follow-up with a nephrologist.

## Evidence

There are few studies that describe the PD method as an immediate treatment option in patients without functioning vascular access and only two small studies that compared unplanned start of HD versus PD.[46-47] These studies showed that there was no significant difference in the mortality rates between the two methods.

Lobbedez et al.[47] followed 60 patients who started unplanned dialysis for a 2-year period. Among the patients who started on PD, only two had mechanical complications after catheter implantation and showed no significant difference in mechanical or infectious complications when compared with patients who had 'rest time' post catheter insertion. There was no significant difference in patient survival between the two unplanned dialysis methods (78.8% versus 82.9%, p=0.26).

Koch et al.[46] evaluated 57 incident patients in unplanned HD and 66 in unplanned PD. HD patients had a higher rate of bacteraemia than PD patients in the first 6 months of dialysis (21.1% versus 3%, p<0.01), which was associated with the use of CVC as initial access. However, there was no significant difference in the mortality rates between the two methods.

Danish data support the idea that early unplanned PD is associated with lower risk of infectious complications compared to the incident HD patients using CVC.[48] The authors noted that there was a higher number of cases of catheter-related mechanical complications in patients starting unplanned PD compared with those who had 'rest time' after implantation of the peritoneal catheter, although it did not affect the method or patients' survival.

Since July 2014, we have offered PD as urgent start for chronic patients. We evaluated our first year of experience[51] concerning technique and patient survival on unplanned PD in the first 90 days. In this prospective study we described how acute PD was initiated right after (<48 hours) PD catheter placement using HVPD until metabolic and fluid control were achieved. After hospital discharge, patients were treated by intermittent PD on alternate days at the dialysis unit until family training. Fifty-five patients were included from July 2014 to July 2015. The mean age was 57.7±19.2 years, diabetes was the main aetiology of CKD (40.6%), and uraemia was the main dialysis indication (54.3%). Metabolic and fluid controls were achieved after five sessions of HVPD and patients remained in intermittent PD for 23.2±7.2 days and received 11.5±3.1 intermittent PD sessions. Peritonitis and mechanical complications occurred in 14.2% and 25.7% of patients, respectively, within 90 days. The mortality rate was 20% and technique survival was 85.7% in the first 90 days. The chronic PD programme presented growth of 79%. We concluded that the concept of urgent start on chronic PD may be a feasible, safe, complementary alternative to HD, and a tool to increase the PD

penetration rate among incident patients starting dialysis therapy. Although data on unplanned initiation of PD are scarce, they indicate that mortality is the same or even better than in cases of unplanned initiation of HD, and that the number of infectious complications including bacteraemia appear lessened.[45-47,51] It is clear that PD is a suitable method for unplanned dialysis patients and that acute automated PD may help nephrologists to deal with patients without any permanent vascular access at dialysis initiation.

To conclude, our observations suggest that the PD modality may be a feasible and safe alternative to HD not only in planned, but also in urgent start. Moreover, the concept of unplanned start on chronic PD may be a tool to increase the PD penetration rate among incident patients starting chronic dialysis therapy. Urgent-start PD is an option and should be offered in an unbiased way to all patients without contraindications to PD starting unplanned dialysis.

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# PATIENT INSIGHT AND TREATMENT EXPECTATIONS IN ERECTILE DYSFUNCTION

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**Disclosure:** Hartmut Porst is an Investigator, Speaker, and Consultant for Auxilium, Eli Lilly, Menarini/Berlin Chemie, and Sanofi.
**Received:** 11.01.16 **Accepted:** 29.03.16
**Citation:** EMJ. 2016;1[2]:34-41.

## ABSTRACT

In the literature, a strong preference towards pharmacological management with oral phosphodiesterase type 5 (PDE5) inhibitors has been demonstrated in men with erectile dysfunction (ED) versus other methods. However, following pharmacological management, a large proportion of men with ED discontinue treatment prematurely. Therefore, a better understanding of the expectations from, and demands on modern ED management from both the patients and their partners is needed in order to identify factors that may improve outcomes, patient adherence, and patient satisfaction with therapy. Thus, we will present new findings on patient and partner satisfaction and preferences, and discuss how the current pharmacological armamentarium can answer these needs.

Keywords: Erectile dysfunction (ED), patient preference, treatment adherence, quality of life, sildenafil, vardenafil, tadalafil, avanafil, oral phosphodiesterase type 5 (PDE5) inhibitors.

## INTRODUCTION

Erectile dysfunction (ED) is a self-reported condition that is defined as the persistent 'inability to achieve or maintain an erection sufficient for satisfactory sexual performance'; it is the main complaint in male sexual medicine.[1] While sexual performance and overall evaluation of sex life satisfaction are highly subjective, partner-related, multi-factorial, and subject to a high inter-individual variability, ED may affect physical and psychosocial health, and therefore may result in poorer sexual intimacy and a lower quality of life.[2-5]

Both ED incidence (26 new cases per 1,000 men each year)[6] and prevalence, as well as ED severity are strongly correlated with age, with a worldwide prevalence of 37–52% in adults aged ≥40 years.[3,7-12] As the pathophysiology and aetiological factors contributing to ED are widely documented in the literature and well known in the medical domain, this review will focus on the insights and treatment expectations of patients alone. Pharmacological management with oral agents is the first-line therapeutic modality, as opposed to other methods, such as vacuum erection devices, intraurethral alprostadil, or intracavernous self-injection therapy with vasoactive drugs.[12-16]

Following pharmacological management however, a large proportion of men with ED discontinue treatment prematurely. Therefore, a better understanding of the expectations from, and demands on modern ED management both from the patients and their partners is needed in order to identify factors that may improve patient outcomes, adherence, and satisfaction with therapy.

This review will present new findings on patient and partner satisfaction and preferences, and discuss how the current pharmacological armamentarium can comply with these needs.

## PATIENT INSIGHTS AND TREATMENT EXPECTATIONS: RESULTS FROM A LARGE ONLINE SURVEY

Burri and Porst[17] recently conducted a large online survey to better understand patients' needs and expectations regarding sexual activity and ED management. The study was conducted within an online consumer panel contacted via email.

The aim was to collect data via recruitment of sexually active heterosexual individuals aged 30–75 years, who were either healthy or suffering from ED.

Diagnosis of ED was based on the abbreviated form of the International Index of Erectile Function (IIEF-5), which is a validated global assessment questionnaire, commonly used to evaluate male sexual function in clinical research.[18,19] The ideal cohort for the study would have included 80% of individuals with ED, with one-third from each treatment group (treatment-naïve patients [NGs], previously-treated patients [PTGs], and currently-treated patients [CTGs]), and 20% healthy men (HG). While reporting bias may have affected the results given the sensitive nature of the topic, this survey still provides a large-scale picture of ED patient expectations.

## Patient Population

The final patient population was composed of 1,534 men with a mean age of 46±10.9 years (range 30–75), of which 73% (n=1,124) had a history of ED (47%, n=529 NG).[17] In most ED patients (53%, n=590), the condition was of mild severity according to the IIEF-5, with an average disease duration of 49±42.5 months.

## Importance of Sexual Activity

Sexual activity was evaluated as important (41%, n=622) or very important (37%, n=575) in the majority of patients, regardless of age.[17] However, NG patients considered sexual activity significantly less important, as compared with CTG patients (chi-square, $\chi^2$=10.15, p=0.02). To put these findings into perspective, the FEMALES study focussed on the sexual experiences and perceptions of the female partners of men with ED (n=293).[20] Significantly fewer women reported satisfaction with their sexual relationship after their partner developed ED, compared with before (85% versus 39%, p<0.001).

## Importance of Erectile Function

Within the cohort, men considered 'maintaining an erection until the partner reaches orgasm' to be most important aspect of sexual intercourse.[17] The importance of the occurrence of multiple episodes within one sexual encounter seemed to be correlated with age, with younger men shifting towards this aspect, while older men considered 'maintaining an erection until the partner reaches orgasm' to be a higher priority (r=0.08, p=0.006).

## Aspects Related to a Fulfilled Sex Life

The most frequently described aspects contributing to a fulfilled sex life were 'being able to please their partner', 'feeling pleasure', and 'partner involvement'. Similar to erectile function, the level of importance of all the aspects was highest in the HG, then in the CTG, and lowest in the NG groups.[17] In the FEMALES study, decreases in the frequency of orgasms were significantly related to the severity of their male partner's ED (p<0.01).[20]

## Spontaneity and Naturalness of the Encounter

The 'not having to plan' and 'engaging in sex whenever he wants' aspects were considered as less important than the previously mentioned aspects in the overall sample, but were considered more important by younger men (Pearson correlation [$r_p$]=−0.06, p<0.05; $r_p$=−0.61, p<0.05, respectively).

Not having to plan the exact timing of intercourse is nevertheless a relevant aspect of a couple's sex life. In the FEMALES study, the latter aspect was a relevant topic, with 34% of the females and 30% of the males stating that they did not have a set pattern regarding predictability of sexual activity.[20] Similarly, 61% of females and 52% of males denied advance aspiration of sexual activities, underlining the importance of spontaneity in their sex life (Figure 1).

## Ideal Onset of Action

Thirty-eight percent of men (n=584) considered an ideal onset of action to be of 'about 15 min', giving them 'the ability to respond immediately to the partner's sexual wishes and requests' and 'allowing a certain degree of spontaneity'. This finding was observed across all ED treatment groups (Figure 2). A further 28% of men (n=423) considered that an ideal onset of action was somewhere 'between 15 and 30 min', and for 34% of the sample (n=527) 'between 30 and 60 min'. None of the respondents considered an onset of 'more than 60 min' to be adequate or desirable.

## Ideal Duration of Effect

Most men with ED (96%, n=1,078) considered a duration of up to 4 hours to be desirable, and 48% (n=536) of men considered 6–12 hours to be adequate. Conversely, approximately 71% of men in the ED groups (n=798) considered a duration of >12 hours to be too long.

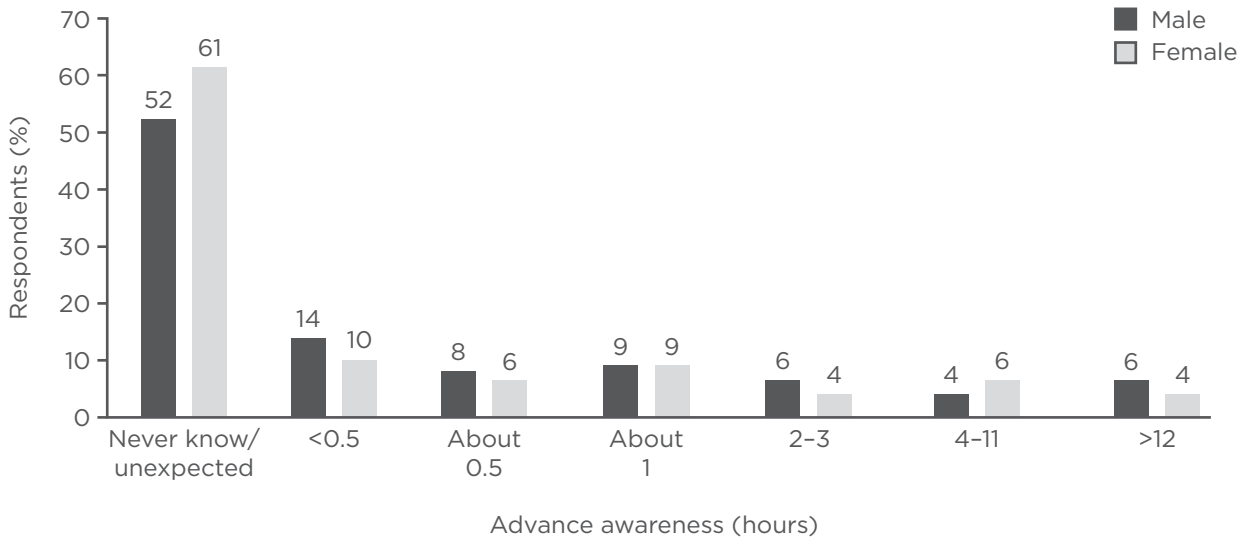



**Figure 1: Male–female reports of advance awareness of sexual activity.[20]**

## Ideal Erectile Dysfunction Therapy Characteristics

The findings from this survey could reflect the key pharmacokinetic features to aim for in an ideal ED drug according to each patient's expectations. Oral therapy for ED is generally expected by patients to boast a high efficacy, with a fast onset of action and a desirable window of efficacy. In this survey, results showed that once the basic aspects of ED management are satisfied, namely achieving and sustaining an erection, patients look for qualitative versus quantitative factors associated with a fulfilled sex life, such as spontaneity and being able to please their partner. The corresponding pharmacokinetic features that patients prioritised to meet their wishes and expectations to improve their satisfaction in sexual life were a fast onset of action of approximately 15 min and a reasonably long duration of efficacy between 6 and 12 hours.

# OVERVIEW OF THE CURRENT MANAGEMENT OF ERECTILE DYSFUNCTION

ED management is aimed at restoring the capacity to initiate and maintain a rigid penile erection, enabling the patient to perform satisfactorily during sexual intercourse. It comprises pharmacological management with oral phosphodiesterase type 5 (PDE5) inhibitors, penile self-injection programmes with vasoactive drugs, intraurethral therapy, vacuum erection devices, and penile prostheses for men.[12,21] Because of the great variety of underlying aetiologies for ED, a successful initiation of medical therapy is highly dependent on the patient's characteristics and comorbidities.[12,21]

# ORAL PHARMACOLOGICAL MANAGEMENT OF ERECTILE DYSFUNCTION

PDE5 inhibitors inhibit the PDE5 enzyme involved in the catabolism of cyclic guanosine monophosphate, which is in turn responsible for the vasodilation mechanisms of penile erection.[22] PDE5 inhibitors are easy to use and have a demonstrated efficacy in the number and duration of erections in patients with ED, with a favourable benefit-to-risk ratio and a low rate of side-effects. As such, oral PDE5 inhibitors have been established as the first-line medical therapy for ED.[16]

In the FEMALES study, the proportion of women who experienced sexual desire, arousal, and orgasm 'almost always' or 'most times' was significantly higher when their partner was currently treated with a PDE5 inhibitor ($p<0.05$).[20]

## Sildenafil

Sildenafil (Viagra®, approved by the European Medicines Agency [EMA] in 1998) was the first oral PDE5 inhibitor approved to treat ED and has been the subject of many clinical trials.[23] The recommended dose is 50 mg, taken as needed approximately 1 hour before sexual activity. Based on efficacy and tolerability, the dose may be increased to 100 mg or decreased to 25 mg. The maximum recommended dose is 100 mg.[24]

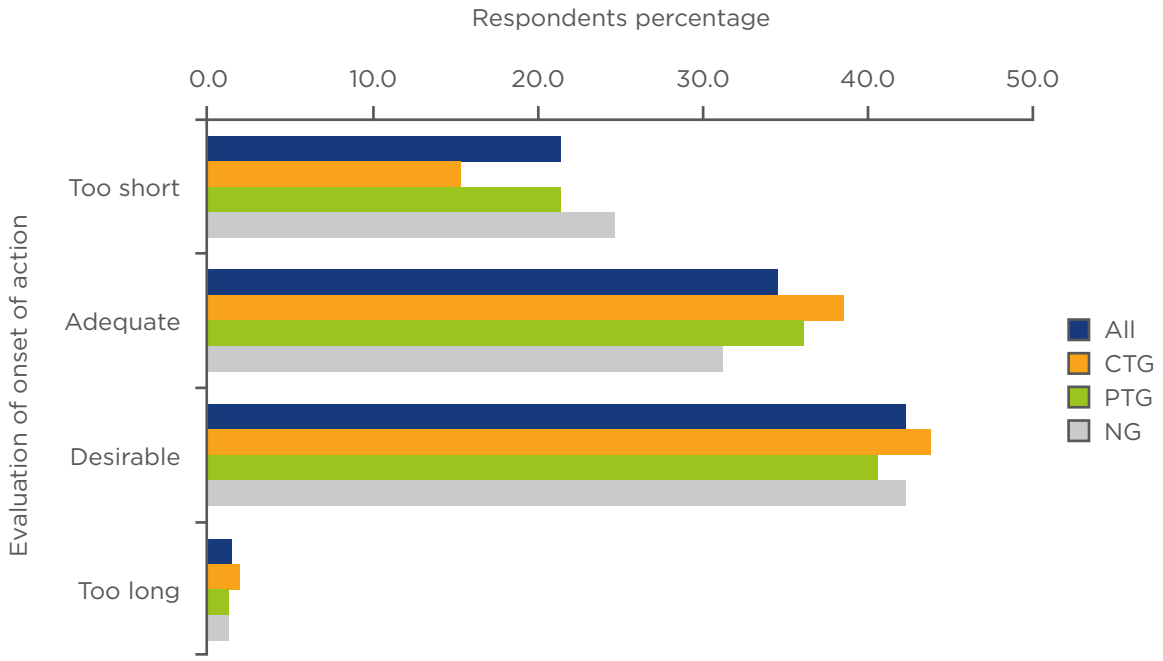



**Figure 2: Evaluation of onset of action of about 15 minutes by currently-treated (CTG, n=298), previously-treated (PTG, n=297), and naïve erectile dysfunction (ED) patients (NG, n=529).[17]**

Adverse events (AEs) include flushing (12%), headache (11%), dyspepsia (5%), and visual disturbances (3%), but sildenafil is not significantly associated with serious cardiovascular events or death.[25] It should be noted that the onset of action can be delayed after a heavy, fatty meal or alcohol intake due to prolonged absorption. Co-administration with non-selective alpha-1 blockers may cause symptomatic hypotension in patients using sildenafil intermittently.

## Vardenafil

While no head-to-head studies have been conducted to compare the efficacy of vardenafil (Levitra®, approved by the EMA in March 2003) with sildenafil, it seems the former presents a similar onset, duration of action (up to 4 hours), and safety profile compared to that of sildenafil.[21,26-30]

The recommended dose of vardenafil is 10 mg, taken as needed approximately 25–60 minutes before sexual activity. Based on efficacy and tolerability, the dose may be increased to 20 mg or decreased to 5 mg.[31] Vardenafil is available as a film-coated tablet or in a new formulation as a orodispersible tablet, which could generate a more rapid onset (≤30 minutes) of action.[32-34] As with sildenafil, administration with a high-fat meal or alcohol consumption may delay the absorption with the film-coated formulations.

## Tadalafil

Tadalafil (Cialis®, approved by the EMA in February 2003) has a completely different chemical structure than the first two drugs, providing similar efficacy outcomes and a well-tolerated safety profile, but a longer duration of action.[35] With a plasma half-life of 17 hours, tadalafil has the longest window of opportunity of up to 36 hours.[36-38]

The recommended dosing is of 10–20 mg prior to anticipated sexual activity, with or without food. In those patients in whom tadalafil 10 mg does not produce an adequate effect, 20 mg might be tried. It may be taken at least 30 minutes prior to sexual activity. A lower dose of 5 mg is available for once-daily dosing and has been approved both for the treatment of ED and benign prostatic hyperplasia/lower urinary tract symptoms.[39-41] Tadalafil absorption is not affected by food intake (namely, fatty meals) or alcohol consumption. AEs reported with tadalafil are comparable to the other two PDE5 inhibitors with the exception of myalgia and back pain, which are observed more often.[16,21]

## Avanafil

Avanafil (Spedra®, approved by the EMA in June 2013) is the newest available PDE5 inhibitor and is considered a second-generation agent due to its enhanced PDE5 selectivity as compared with the

first three compounds.[42] Avanafil has a more rapid onset of action (≤15 minutes) with a similar efficacy, but the main advantage of avanafil in comparison to the first-generation PDE5 inhibitors is its improved safety profile, due to its high selectivity for PDE5.[43-49] The recommended dose is 100 mg taken as needed approximately 15–30 minutes before sexual activity. Based on individual efficacy and tolerability, the dose may be increased to a maximum dose of 200 mg or decreased to 50 mg.[50]

The rapid onset of action has been evidenced by a randomised, double-blind, placebo-controlled registered clinical trial involving 646 patients with ED over a 12-week treatment period (67% and 71% successful intercourse attempts with 100 mg and 200 mg avanafil compared with 27% with placebo, respectively; Figure 3).[51]

In a newly-published, randomised, double-blind, placebo-controlled, 12-week study (4-week run-in and 8-week treatment), 145 men were assigned to placebo, 147 to avanafil 100 mg, and 148 to avanafil 200 mg on demand.[52] Successful intercourse attempts within approximately 15 minutes of dosing were significantly higher with avanafil 100 mg (mean 25.9%) and 200 mg (mean 29.1%) versus placebo (mean 14.9%, p=0.001 and p<0.001, respectively). A statistically significant difference between avanafil and placebo was observed in the average per-subject proportion of successful intercourse attempts as early as 10 minutes in the 200 mg group and 12 minutes in the 100 mg group (Figure 4).

The average duration of action of avanafil has been reported as beyond 6 hours in some subjects.[51] Treatment-emergent AEs were similar but generally lower compared with the other PDE5 inhibitors. AEs reported with avanafil include headache, flushing, and nasal congestion. Although avanafil clinical trials have been conducted without any kind of restrictions on food and alcohol, high-fat meals could delay its rate of absorption into the plasma.[49,50]

## Safety Profiles of PDE5 Inhibitors

AEs occurring with PDE5 inhibitors are generally mild or at best modest, and are mostly transient and self-limited.[16,21,30,36,53-55] As stated previously, the most commonly reported AEs are headache, flushing, dyspepsia, nasal congestion, and dizziness. All PDE5 inhibitors are contraindicated with the use of nitrates or nitric oxide donors of any form due to the risk of severe and sometimes life-threatening hypotension. Moreover, PDE5 inhibitors are to be used with caution with non-selective alpha-blockers and potent CYP3A4 inhibitors.[56]

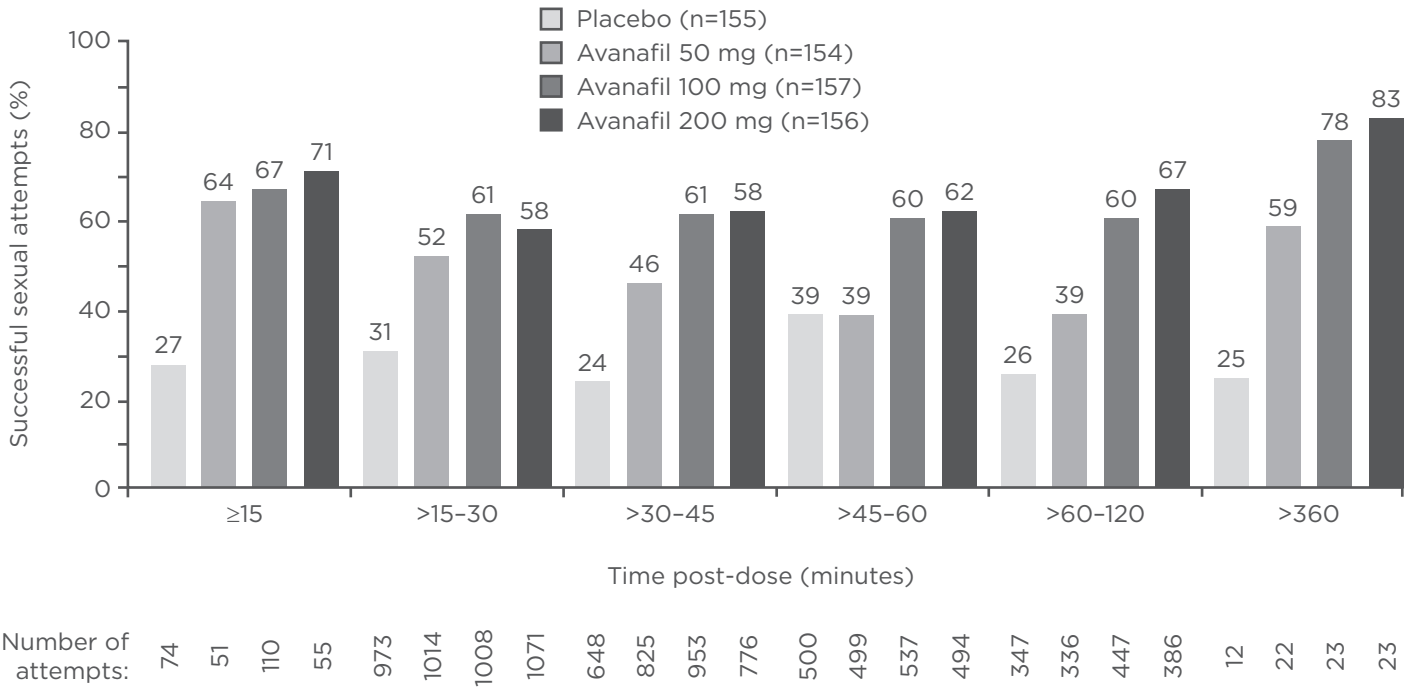
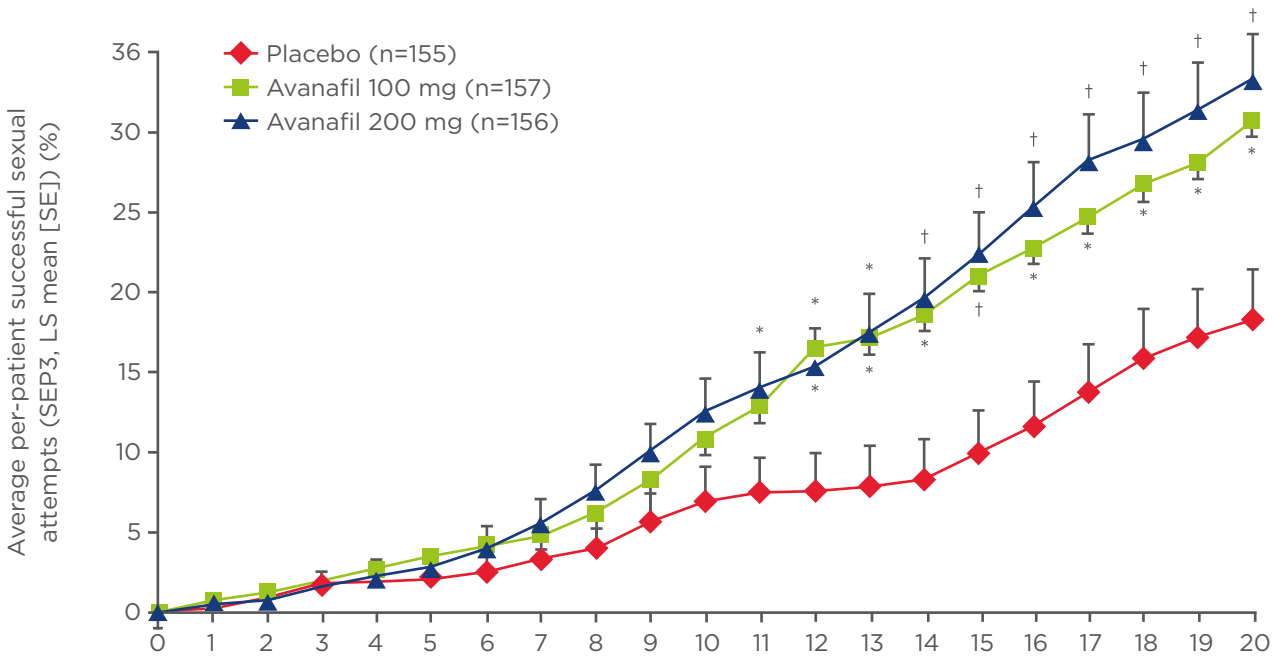



**Figure 3: Successful intercourse by time interval, from dose to attempt. Sexual attempts in which subjects were able to maintain an erection of sufficient duration to have successful intercourse by post-dose time interval.[51]**




Number of subjects with at least one success at the timepoint:

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Placebo | 2 | 5 | 8 | 8 | 10 | 14 | 17 | 21 | 30 | 33 | 34 | 36 | 37 | 42 | 5 | 51 | 55 | 56 | 59 | 62 |
| Avanafil 100 mg | 7 | 11 | 14 | 16 | 22 | 23 | 28 | 36 | 42 | 53 | 56 | 65 | 66 | 70 | 74 | 76 | 80 | 84 | 85 | 90 |
| Avanafil 200 mg | 4 | 6 | 12 | 16 | 20 | 23 | 29 | 36 | 40 | 49 | 56 | 59 | 63 | 68 | 79 | 83 | 83 | 85 | 88 | 90 |

**Figure 4: Sexual attempts during the 8-week treatment period in which intent-to-treat subjects maintained erection of sufficient duration for successful intercourse by time since dose administration.[52]**
*p<0.05 versus placebo. †ANCOVA p<0.001 versus placebo.
LS: least squares; SE: standard error.

Avanafil seems to be associated with reduced incidence of common AEs as compared with the other agents, but head-to-head trials or longer duration studies on the safety of avanafil are needed to confirm this perceived advantage.[12,49]

## RATIONALE FOR CHOICE OF THERAPY: ADDRESSING PATIENT EXPECTATIONS WITH THE CURRENT ARMAMENTARIUM

Sildenafil, vardenafil, tadalafil, and avanafil are mostly equivalent in terms of efficacy profiles, but different pharmacokinetic properties can allow physicians to choose the most appropriate drug according to the patient's/couple's characteristics and expectations.[16,46,57] While all four compounds provide a similar efficacy in regards to the rates of successfully completed intercourse (SEP 3 data) in men with ED, there is no direct head-to-head data from double-blind multicentre studies comparing the efficacy or tolerability of PDE5 inhibitors.[21,57]

In 2009, the American College of Physicians recommended that the choice of PDE5 inhibitor be based on patient's preferences, costs, ease of use, and desired onset and duration of action, as well as AEs.[58] The 2015 guidelines on male sexual dysfunction published by the European Association of Urology recommend that the choice of drug should be based on the frequency of intercourse and the short or long-acting properties of the options, while highlighting that patients should be aware of these characteristics and how to use them.[12]

The differences in pharmacological characteristics and pharmacokinetic profiles, with different onset and duration of action parameters, differentiate PDE5 inhibitors and contribute to ED therapy more 'individually tailored' to the couple's needs. While sildenafil, film-coated vardenafil, and tadalafil should be taken 1 hour before sex, orodispersible vardenafil and avanafil can be taken only 30 and 15 minutes before sex, respectively, which is of particular interest for those couples who have a spontaneous sex life.

Moreover, daily low-dose tadalafil can be recommended in men seeking to eliminate

concerns regarding the preservation of spontaneity in their sex life and in this context the onset or duration of action of a drug. However, this represents a costlier treatment option compared with on-demand regimens.

## CONCLUSIONS

There is no doubt that avanafil is not only an interesting addition to, but a real enrichment of the class of PDE5 inhibitors, due to its rapid onset, reasonably long duration of action, and superior safety profile thanks to its high selectivity. These pharmacokinetic and pharmacodynamic advantages will likely improve patient compliance and couples' treatment satisfaction, with fewer treatment discontinuations. Therefore, avanafil may represent a valid option for all those patients who are not satisfied with the pharmacokinetic properties and the rate of side effects associated with the three older PDE5 inhibitors.[12,16]


## Acknowledgements

Medical writing assistance was provided by Dr Caroline Charles (Scilink Medical Writing, Biarritz, France).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# THE MULTIFACTORIAL BACKGROUND OF EMERGING VIRAL INFECTIONS WITH NEUROLOGICAL MANIFESTATIONS

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 01.03.16 **Accepted:** 21.03.16
**Citation:** EMJ. 2016;1[2]:43-49.

## ABSTRACT

The events of the past year have highlighted the continuing importance of emerging virus infections on the diagnosis and treatment of neurological disease. This review focusses on clarifying the effects of the multiple overlapping factors that impact emergence, including viral richness, transmission opportunity, and establishment. Case studies of the West Nile, chikungunya, and Zika viruses are utilised to illustrate the dramatic effects of expansion in the range and geographical distribution of emerging infectious disease, the acquisition of new virus vectors, and of increasing human anthropogenic factors such as global transport, climate change, and mosquito abatement programmes on the regional spread and clinical consequences of emerging infectious disease.

Keywords: Emerging infectious disease (EID), neurological disease, emerging viruses, Zika virus (ZIKV), chikungunya virus (CHIKV), West Nile virus (WNV), microcephaly, Guillain–Barré syndrome.

## INTRODUCTION

The increasing occurrence of virus-induced emerging infectious disease (EID) is a significant global health concern.[1,2] EID is characterised by the presence of viruses that display either new transmission or increasing disease incidence, geographic range, or pathogenicity.[3,4] Approximately 60–75% of human EIDs are zoonotic,[4,5] thus the epidemiology of vectors and hosts represents a critical component of emergence. At one level emergence can be attributed to virus adaptation, but EID is increasingly impacted by anthropogenic effects as varied as changes in land use (e.g. deforestation), climate change, immigration, and the ease of human and product global transportation.

Infections with emerging viruses often profoundly impact the nervous system. Over the past decade, approximately 50% of EID in humans have exhibited some level of neurological involvement, often with life-threatening consequences.[6] We need look no further than the recent outbreak, rapid spread, and neurological impact of Zika virus (ZIKV) in the Americas to appreciate the magnitude of this concern.[7]

Comprehensive overviews of viruses that cause neurological disease have been published in recent years.[8-10] Nonetheless, the direct consequences of viral infection within the nervous system are often difficult to diagnose clinically as symptoms may overlap with more common, non-infectious causes. Clinical diagnosis is also frequently hampered by the diversity of emerging viruses, their mode of transmission and latency, a knowledge gap in the biology of emerging viruses, and the corresponding lack of high-level clinical facilities and diagnostic tests that enable precise laboratory-based distinction of viruses and/or virus subtypes.

Viruses that were once geographically widespread and thought to be controlled, such as poliomyelitis, may re-emerge, and viruses that are endemic to one region may spread episodically to another, as has been the case with the Ebola virus. The regional origins of emerging infections contribute

to this dilemma, as it is common to assume that such infections only occur in exotic locations. However, it has been shown that the highest concentration per area of EID occurs between latitudes of 30–60° north and 30–40° south,[5] with predicted hotspots in tropical countries of Asia, Latin America, and Africa due to the abundance of vector-borne pathogens, increased animal zoonosis, and human population density. This constellation of effects contributes to a general lack of awareness and preparedness for the potential spread and corresponding neurological effects of emerging viruses.

The earliest phase of emergence is dependent on virus transmission, which is governed by the essential drivers of viral richness (genetic diversity, saturation frequency of host infection, and climate adaptability), transmission opportunity (population density, alternate host availability, geographic vector restriction, host mobility, and other unique exposure frequency), and establishment (ease of human transmission and herd immunity).[11] Perhaps the best example of richness as a driver of EID is the high mutation and recombination rates inherent to retroviruses (e.g. human immunodeficiency virus) and orthomyxoviruses (influenza viruses), respectively. However, many other viruses also display relatively high rates of mutation and adapt rapidly to new environmental conditions, often in spatially disparate locations.[12,13] EID is also linked to changes in transmission opportunity through either expansion of virus host range and/or geographical distribution. This is particularly evident in that many viruses do not survive for long periods of time outside of their hosts. As a result, transmission is often heavily influenced by the proximity of hosts and/or vectors. Several recent EID outbreaks of viruses transmitted by arthropod vectors (arboviruses) provide illustrative case studies of the interplay among these biological and anthropogenic factors in driving the rapid geographic spread of EID.

## THE IMPACT OF EXPANDED HOST RANGE ON EMERGING INFECTIOUS DISEASE

The spread of West Nile virus (WNV) in the USA provides a classic example of the impact of host range expansion on EID transmission. WNV is a mosquito-borne *Flavivirus*, first isolated in 1937 in the West Nile region of Uganda. Over the intervening years, WNV was detected in sporadic EID outbreaks of encephalitis, meningitis, and more rarely, acute flaccid paralysis, throughout North Africa and Eastern Europe, with more recent discrete reports in Romania (1996), Morocco (1996), Tunisia (1997), Italy (1998), and Israel (1998). In contrast, WNV was not detected in the USA prior to 1999. Indeed, epidemic arbovirus infections in humans were rare in the USA over the past century, with the exception of a major epidemic of St Louis encephalitis virus in the 1970s.[14,15]

However, in 1999, a cluster of neurological disease involving 62 infected subjects, with 59 requiring hospitalisation, were reported in New York City. Epidemiological analysis coupled to antigenic and genetic sequence mapping definitively identified WNV as the causative agent.[15] Subsequently, WNV expanded geographically at an alarming rate within the USA, such that by 2003, over 2,500 cases of WNV neuroinvasive disease were reported. The estimated total number of WNV infections in the USA now exceeds 1 million, encompassing the 48 contiguous states.[16] The majority of WNV infections are asymptomatic; approximately 20% present as fever and myalgia, and the incidence of serious neurological disease from WNV infection remains relatively constant at approximately 0.7%.[16-18] Documentation of infection is not straightforward as antigenic cross-reactivity among *Flavivirus* proteins is the norm, and thus disease symptoms must be coupled to the presence of either antiviral IgM in acute-phase serum or cerebral spinal fluid (CSF), an IgG titre shift of 4-fold or higher between acute and convalescent phases, and/or the direct demonstration of virus antigen or RNA in serum, CSF, or tissue.[15]

Determining the origins of WNV in the USA presents a challenge that remains speculative to this date. The lifecycle of WNV includes birds as the principle host, with the spread to humans and other species driven primarily by mosquitoes of the *Culex* genus, which are found throughout the USA. Although several models have been proposed to explain the origins of the initial cases in the USA, the most plausible rests with expansion of the avian host range. Genetic analysis of the virus isolated from patients demonstrated that the 1999 WNV isolate in New York City closely resembled the virus isolated from two avian species in 1998, harvested at the time of a local WNV epidemic in Tel Aviv, Israel.[19-21] Although WNV is rarely fatal in avian species, both the outbreak in Israel and New York were characterised by significant avian deaths.[15] In addition, birds can support

exceptionally high levels of viraemia for several weeks with titres of up to $10^{11}$ plaque forming units (pfu)/mL reported, whereas virus titre in humans is transitory and rarely exceeds $10^{2-3}$ pfu/mL.[22] Lastly, the spread of WNV throughout the USA was attributed to high virus titre in a relatively small number of common avian species such as robins (*Turdus migratorius*), blue jays (*Cyanocitta cristata*), and house finches (*Haemorhous mexicanus*).

Collectively, these observations suggest that the most likely origin of WNV in New York City resulted from the introduction of infected birds to the USA, rather than mosquitoes or infected humans. This might have occurred either by transcontinental migratory patterns, or by the introduction of infected birds as components of commercial or other transport.[24] Once in the USA, it is likely that the virus percolated within common avian species such as crows, where multiple deaths were observed, reaching both high infection penetrance and titre, which in turn promoted efficient mosquito transmission to humans during the exceptionally hot summer of 1999.[15,24]

## VECTOR BIOLOGY AND HUMAN FACTORS ENHANCE THE ORIGINS OF EMERGING INFECTIOUS DISEASE

Chikungunya virus (CHIKV) provides an important example of the balance among virus genetics, vector diversity, and anthropogenic effects in driving the global spread of infection and disease incidence. CHIKV is a mosquito-borne *Alphavirus*, which, while not uniformly neuropathic, has been shown to induce neurological disease in up to 12% of infected individuals in isolated outbreaks.[10,25] The incubation period for the disease is 3–7 days and it typically manifests as encephalomyelitis, radiculitis, Guillain–Barré syndrome, or acute flaccid paralysis.[9,10,26] In children, symptoms may be more severe and include altered levels of consciousness, seizures, and paraplegia.[27] Rare maternal-fetal transmission may also occur in viraemic mothers, with severe fetal encephalopathy observed in 50% of these cases.[27,28]

CHIKV was discovered in 1952 in Tanzania and until the last decade only sporadic outbreaks were reported in Africa and Asia.[29] However, the geographic range of CHIKV has spread dramatically over the past 10 years, including outbreaks in East Africa (2004), islands of the Indian Ocean and Sri Lanka (2005–2007), and an index case for an autochthonous CHIKV epidemic in Northeast Italy that was tracked to a visitor from Kerala, India, where CHIKV infection was widespread (2007).[30] Within the past 24 months, CHIKV has spread to other parts of Europe and rapidly throughout the Americas from Brazil to the USA with >750,000 cases reported in total.[24,31]

Phylogeographic analysis of CHIKV genomes indicates that the recent geographic expansion in the Caribbean and Americas is linked to the Asian genotype, whereas expansion to South Asia represents the Indian Ocean genotype of CHIKV.[32] The virus is primarily spread by *Aedes aegypti* and *Aedes albopictus* mosquitoes; importantly, as the geographic range of CHIKV expanded, the Asian lineage of the virus evolved by accumulation of a single amino acid change (A226V) in the viral E1 protein that enhanced transmission and infectivity in *Ae. albopictus*.[33]

While expansion of vector distribution and transmission were primary drivers of the spread of CHIKV, the globalisation of CHIKV can also be attributed to multiple anthropogenic factors. These include the failure of mosquito control efforts in the Americas, increased vector habitats from growing urbanisation in the tropics, increased tyre trade across regional boundaries in Asia, and greater human transport between Asia, Africa, and the Americas at times of peak vector activity.[34-36] Creating a precise linkage of CHIKV genetics and vector utilisation with the incidence of disease and, more specifically, the detailed characterisation of the neurological effects within each EID outbreak, has proven to be a significant challenge as many of the effected regions have suboptimal health care delivery and/or reporting systems.

## CONFOUNDING EFFECTS OF PUBLIC HEALTH AND EPIDEMIOLOGY PREPAREDNESS ON EMERGING INFECTIOUS DISEASE

The current spread of ZIKV in the Americas represents an ongoing pandemic, the causes and clinical outcomes of which remain hotly disputed. ZIKV is a mosquito-borne *Flavivirus*, first isolated in 1947 from a sentinel monkey in the Zika forest of Uganda, but it is likely that it originated between 1892 and 1943.[37,38] The virus distribution spread slowly over the course of 60 years, coupled to mutation and possibly genetic recombination that enabled adaptation to new vectors. This eventually

led to ZIKV spread to countries in Central, North, and West Africa, as well as Southeast Asia.[38-40] Serological evidence from these outbreaks indicated that approximately 80% of ZIKV infections were asymptomatic with penetrance in 5–10% of the population. Indeed, the relatively benign past nature of ZIKV infection is reinforced by the observation of only 14 confirmed human cases, and no significant morbidity or mortality, from discovery until 2007.[38,40]

This changed dramatically in 2007 with the detection of a ZIKV outbreak on Yap Island in the Federated States of Micronesia. Viral RNA analysis confirmed the presence of ZIKV in 49 individuals, with an additional 59 probable but unconfirmed cases.[41] This outbreak spread to multiple islands within French Polynesia between 2013 and 2014. In this area, there were 294 laboratory confirmed cases and in excess of 20,000 suspected cases of infection.[42,43] Disease symptoms were similar to those described previously including rash, fever, arthralgia, and conjunctivitis. However, a distinguishing clinical outcome was 72 cases of severe neurological symptoms including 40 suspected cases of Guillain–Barré syndrome.[43] Approximately half of these cases were in individuals who displayed illness compatible with ZIKV infection, although in none of these instances was ZIKV infection definitively confirmed by laboratory analysis.

Of increasing concern is the more recent spread of ZIKV to the Americas and Caribbean Islands. Beginning in April 2015, ZIKV was first reported in Brazil and subsequently, as of February 2016, confirmed infections were reported in 31 countries within the region.[44,45] Current estimates of the total number of infections in Brazil alone range from 0.4–1.3 million individuals. Consistent with past outbreaks, the vast majority of ZIKV infections remain asymptomatic and those who present with illness predominantly display symptoms that resolve within 2–6 days. The most troubling aspect of this pandemic lies with coincident data from six countries (French Polynesia, Brazil, El Salvador, Venezuela, Columbia, and Suriname) of an increasing incidence of Guillain–Barré syndrome and, more dramatically, of microcephaly in newborns.[45]

Reports of an increase in babies born with microcephaly first surfaced in 2015 from the state of Pernambuco, Northeast Brazil. Subsequently, similar data was reported nearby in Bahia and Paraíba, with an increased incidence of microcephaly now reported in 23 of the 26 Brazilian states. Though the linkage of microcephaly to ZIKV infection remains speculative, several lines of evidence support this hypothesis. First is the increase in disease incidence: microcephaly was previously rare in Brazil, with an average of 163 cases per year from 2010–2014, but summative reports for 2015–2016 have now identified 5,640 cases of microcephaly and/or central nervous system malformations, 120 of which resulted in death.[45,46] Secondly, ZIKV can cross the placenta; ZIKV RNA has been detected in the amniotic fluid of mothers whobirthed babies born with microcephaly.[47] Thirdly, placental and brain tissue from at least two newborns with microcephaly who died within 20 hours of birth tested positive for ZIKV by reverse transcriptase polymerase chain reaction.[48] Lastly, recent reports indicate that ZIKV can infect and attenuate the growth of human neural progenitor cells in an *in vitro* model system.[49]

Despite these observations, it is likely that reports of microcephaly in Brazil, both before and after the current ZIKV outbreak, are inaccurate. As noted above, prior to 2014, the incidence of microcephaly in Brazil was only 0.5 in 10,000 live births. In other countries where the criteria for diagnosing microcephaly are established and the analysis is conducted by well-trained health professionals, the incidence holds steady at 2–12 in 10,000 live births. Although the ZIKV outbreak is now widespread, >90% of cases in Brazil remain in the Northwest region, encompassing Pernambuco and neighbouring states. This relatively poor region is ill-equipped to deliver the necessary health care during this pandemic and to maintain consistent standards of clinical and laboratory epidemiological outcomes. A more detailed follow-up analysis by Brazilian health authorities on 1,533 cases reported in 2015 indicates that 950 of these should not be classified as microcephaly, and to date, only 30 of the 583 confirmed cases have been linked to congenital ZIKV infection.[45]

Taken collectively, these observations strongly suggest that the rate of microcephaly in Brazil has not increased as significantly as initially reported in the current pandemic. However, whether ZIKV infection induces microcephaly and perhaps Guillain–Barré syndrome remains an open question. It is certainly possible that the large number of ZIKV infections, including the unprecedented levels in pregnant women, may have uncovered a rare but extremely important clinical outcome.

As the majority of neurological disease cases are in medically underserved regions, associated factors such as malnutrition may also contribute to these effects. Lastly, in addition to potential direct pathogenic effects of ZIKV, it is important to highlight that ZIKV antigens share extensive cross-reactivity with other members of the *Flavivirus* genus, such as Dengue virus (DENV), which are also endemic to this region. This creates the potential for immune-mediated effects due to the preexistence of cross-reactive antibodies.

The ZIKV pandemic is likely to continue to spread in the Americas, and possibly to other regions during 2016. Virus transmission primarily occurs via *Ae. aegypti* and *Ae. albopictus* mosquitoes, though there is growing evidence that ZIKV may utilise a wide range of vectors, including eight species of *Aedes* and others within the genera *Anopheles*, *Mansonia*, and *Culex*.[39,50] Additionally, there are now several reports that suggest ZIKV can be sexually transmitted.[51,52] Given that the geographic distribution of *Ae. aegypti* alone now encompasses all continents, the breadth of vectors and transmission routes suggests that ZIKV has the potential to expand much on a global scale.

## CONCLUSIONS AND OUTLOOK

This review highlights several of the major factors that impact the global spread of EID. As outlined in the examples of WNV, CHIKV, and ZIKV, recent EID outbreaks and pandemics result from a combination of factors that simultaneously impact transmission frequency, establishment, and pathogenicity. EID can result from the origin of entirely new viruses, viruses new to a region, or the re-emergence of viruses once thought to be eradicated. Geographic spread can be mediated by utilisation of additional vectors or expansion in a vector's geographic range, addition of new host species, enhanced virus fitness within vectors or hosts, and/or by anthropogenic factors. Indeed, given global climatic changes, variable effectiveness of pesticides in longitudinal control of vector populations, and the ever-increasing reach, volume, and speed of human mobility and trade, the potential for global transmission of EID is greater now than ever before.

The historical challenges faced by Brazil in controlling the *Ae. aegypti* vector highlight the magnitude of this problem. Mosquito abatement efforts in Brazil date to 1946 amid concerns over the spread of yellow fever.[53] Indeed, through the widespread use of dichlorodiphenyltrichloroethane, eradication of *Ae. aegypti* in Brazil was thought to be complete in the late 1950s. However, despite consistent awareness of the need to control mosquito populations, from the late 1960s through to the present, the vector serially reappeared due to the acquisition of drug resistance by new mosquito strains, social and environmental changes resulting from rapid urbanisation, failures in epidemiological surveillance, and inconsistent enactment of governmental policy. More recently, renewed abatement efforts were initiated by Brazil in response to the epidemic spread of DENV, which like ZIKA is transmitted by *Ae. aegypti* and *Ae. albopictus* mosquitoes. Despite this effort, since the detection of DENV in 1981, Brazilian infections have steadily risen with almost 1.5 million cases of DENV reported in 2013 alone. These observations highlight that mosquito vectors are extremely adaptable to human environments and display high reproductive capacity and genetic flexibility. In view of this, an integrated approach with well-coordinated governmental and regional action, and involving a combination of mechanical, biological, and chemical approaches that are linked to a public awareness campaign, is essential.

Despite the seemingly overwhelming variety of emerging viruses and the multiple confounding factors that affect their transmission, and contrary to present circumstances with regard to ZIKV, the future holds promise for greater awareness of, if not control over, the spread of EID. Efforts to block transmission by controlling mosquito populations using genetically modified strains has proven effective in field pilot studies.[54] Many regions of the world are now prepared for, or indeed already conduct routine sampling for emerging viruses. Portable low-cost diagnostic tests for viruses that couple to cellular phone technology are now in development.[55] Through in-country efforts and those of organisations such as the World Health Organization, there is now more rapid and accurate dissemination of the status of EID during outbreaks. Lastly, lower costs of sequencing and the growing abundance of existing virus genomes allows rapid statistical comparisons of emerging viruses across regions which, when coupled with cartography, accelerates the identification of causative viruses. Nonetheless, until each of these components are fully utilised on a global scale, we are likely to witness an acceleration of EIDs, and in that context, awareness of these events is essential when considering perplexing neurological cases.

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# NEXT GENERATION SEQUENCING: A TOOL FOR THIS GENERATION OF NEPHROLOGISTS

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 10.12.15 **Accepted:** 28.01.16
**Citation:** EMJ. 2016;1[2]:50-57.

## ABSTRACT

The emergence of next generation sequencing (NGS) techniques has made the sequencing of whole genomes, transcriptomes, and epigenomes faster and more readily available than previous methods such as Sanger sequencing, which was developed in the 1970s. It is now 10 years since NGS began to revolutionise biological and medical research. Sequencing of RNA provides insights into up or downregulated gene expression patterns and therefore into molecular disease mechanisms. This can lead to the detection of new biomarkers that can be used as diagnostic tools in risk stratification, or even as new therapeutic targets. In nephrology, NGS plays a role in both basic and experimental research, but also in the clinical setting, whereby the diagnosis of innate genetic diseases such as ciliopathies or genetically moderated acquired diseases such as glomerulopathies has improved. NGS enables precise diagnosis and classification of common diseases of the kidneys and urinary tract, aids in both prognostic and predictive decision-making, and in the avoidance of unnecessary therapies. It also plays a role in the risk stratification of disease recurrence after transplantation. NGS is a robust method; however, the performance of NGS is dependent on the method of tissue storage, the extraction of DNA or RNA, and on the sequencing platform itself, as well as on the bioinformatic analyses performed, integration of clinical data, and comprehensive interpretation of the results. The aim of this article is to review and emphasise the importance of NGS as a tool for this generation of nephrologists.

Keywords: Review, nephrology, next generation sequencing (NGS), high-throughput nucleotide sequencing, DNA, transcriptome, epigenome.

## INTRODUCTION

The invention of Sanger sequencing in the late 1970s represented a paradigm shift in the era of modern medicine.[1] The commercialisation of next generation sequencing (NGS), approximately 10 years ago, led to major improvements in both research and the diagnosis of renal diseases. With Sanger's chain termination DNA sequencing method, it is possible to sequence one gene at a time. On the other hand, NGS makes it possible to sequence the whole genome, exome, or predetermined panel of a patient's genes in a single sequencing reaction and in a much more time efficient manner. NGS also has the capacity to characterise all steps of transcription, translation, and methylation of DNA.[2]

The Human Genome Project, which aimed to sequence the whole human genome using Sanger sequencing, took a total of 14 years and was published in 2004.[3,4] The National Human Genome Research Institute subsequently initiated and funded a sequencing technology development programme with the aim of reducing the duration and cost of genome sequencing. This led to a wave of new projects, and finally to the introduction of the contemporary commercial sequencing platforms. The first of these to be released was a method developed by Life Sciences (now Roche) in 2005.[4] It was quickly followed by others, namely a sequencing platform developed by Solexa (now Illumina), in 2006, and the SOLiD platform developed by Applied Biosystems (now Life Technologies) in 2007.[4]

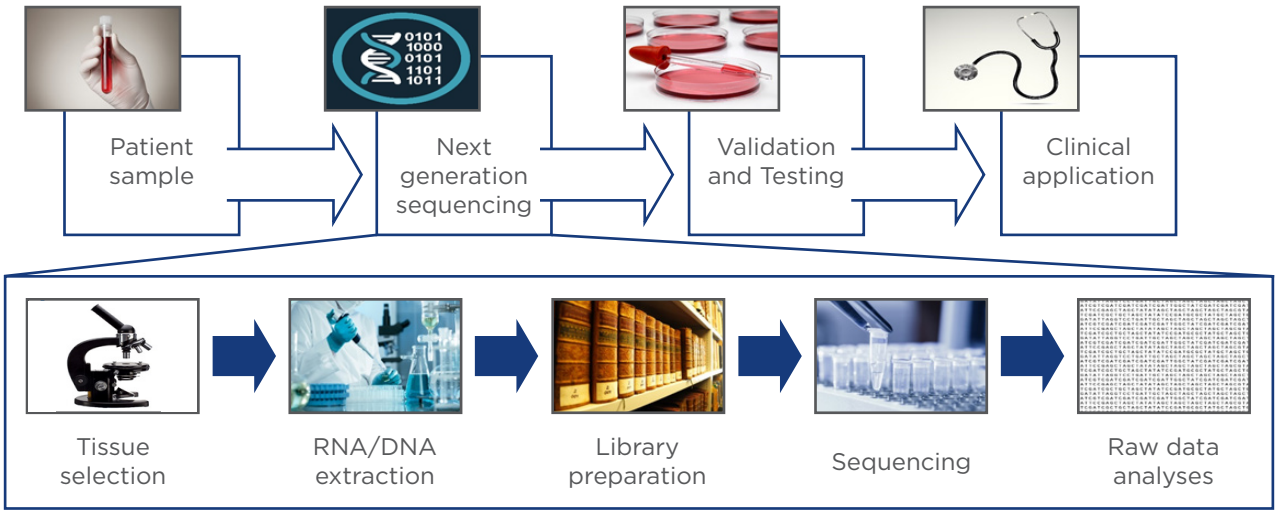


**Figure 1: The upper section displays the application of next generation sequencing (NGS). The lower section illustrates the general workflow of NGS.**

Sequencing techniques within the various platforms are diverse, but all are described as NGS, massively parallel sequencing, or high-throughput sequencing.[2,4-6] The three major advantages of NGS compared with Sanger sequencing are the cell-free system for library preparation, the simultaneous sequencing of potentially millions of reactions in parallel, and the independence of electrophoresis.[4] Compared with other approaches (e.g. microarray), NGS offers the possibility of detecting novel findings that are not based on *a priori* assumptions. Sequencing of DNA enables the simultaneous analysis of the entire genome, which benefits comprehensive genetic diagnostics. Sequencing of RNA gives insight into up or downregulated expression patterns and therefore clarifies molecular disease mechanisms and altered pathways. This can lead to the detection of biomarkers that can be used as diagnostic or stratification tools, or as targets for therapies.[2,7] NGS is a technology that is under continuous development. The vast volume of new data provides great opportunities, but also long lists of gene variations of uncertain significance. Efforts to standardise the analysis of sequencing results are ongoing. This includes the sharing of data and integration of clinical parameters to bring NGS closer to clinical requirements. This requires worldwide collaboration between genetic researchers, bioinformaticians, and nephrologists.[7] Figure 1 illustrates the general application of NGS.

Through improved diagnostics with greater effect on decision-making within both inherited and acquired kidney diseases, NGS helps to improve patient management. In this review, we elaborate on the role of NGS for diagnostic, prognostic, and predictive measures in inherited and acquired kidney diseases, transplantations, and epigenetics. We emphasise the importance of NGS for today's generation of nephrologists.

## TECHNICAL ASPECTS OF DNA AND RNA SEQUENCING

### Tissue Storage

The robust performance of NGS depends both on the technical aspects of tissue storage and nucleotide extraction methods, as well as on the NGS technique itself.[2] Gold standard tissue storage for subsequent sequencing of extracted DNA or RNA is fresh frozen samples. As an alternative, an RNA stabilising solution can be used as a more practical alternative for the storage of fresh frozen tissues. The challenge is that most tissue specimens in pathological archives are formalin fixed and paraffin embedded (FFPE). FFPE tissue is inferior when it comes to molecular analysis as compared with fresh frozen specimens, especially because of the reduced yield and quality of isolated nucleotides. This is due to the process of fixation, embedding, and molecular modification, especially cross-linkage of nucleotides with formalin.[8] However, we have shown that the results of messenger RNA sequencing of pairwise FFPE samples, stored with high quality stabilising solution, yields similar results, thus demonstrating the feasibility of NGS from FFPE tissues.[9]

**Table 1: Sequencing platforms and characteristics.**

| Sequencing platform | Characteristics |
|---|---|
| Illumina technology | On a flow cell, fragments of double stranded DNA denaturated into single stranded DNA are amplified into clusters. During the reading process, the respective nucleotides are added, making this a 'sequence by synthesis' method. The nucleotides are reversible terminators and fluorescent. |
| Ion torrent technology | The reading of the sequence depends not on the emission of fluorescence, but on the emission of protons, which are detected by an ion sensor. Within this method, no amplification of fragments occurs.[2] |
| Pacific bioscience technology | This technique consists of a single molecule real-time sequencing technology. Thus the sensors are able to detect one single molecule. The respective terminated nucleotides are added and recorded in real time.[2] |
| SOLiD technology | Within this technique, a primer is linked to an adaptor. Labelled octamers are added to the primers and sample, and compete for ligation to the primer, whose fluorescence is then read. Thereafter, the octamer is cleaved until the next cycle.[4] |

These findings have been confirmed in a recent publication by a group from the USA.[10]

### DNA and RNA Extraction

There are currently commercially available kits that enable the extraction of DNA and RNA from fresh frozen tissues and from FFPE samples. Using these, it is possible to extract nucleotides from leukocytes isolated from whole blood and from kidney biopsy sections. Laser capture microdissected (LCM) tissues are particularly interesting for RNA extraction, as they allow the selective analysis of gene expression patterns, for example in the analysis of glomeruli in particular glomerulopathies. We have also shown that it is possible to extract RNA of sufficient quality and quantity from a single kidney biopsy and from around 100 LCM FFPE glomerular cross-sections to enable NGS.[11]

### Next Generation Sequencing Techniques

Despite the extensive improvement over Sanger sequencing, the basis of NGS platforms remains a polymerase.[12] Common commercial sequencing platforms are given in Table 1.[2,4] Before NGS is performed, the extracted nucleotides have to be processed to produce a copy DNA library through a library preparation method specific to the particular platform and sequencing approach.[2]

## ROLE OF NEXT GENERATION SEQUENCING IN INHERITED KIDNEY DISEASES

### Detection of Novel Gene Mutations

With whole exome sequencing, it is possible to sequence the entire protein-coding region of the DNA. This method has the capacity to detect novel disease causing genes. In a recent publication, researchers sequenced the whole exome of a group of children with increased renal echogenicity. Causative mutations, in this case for nephronophthisis (NPHP), Alport syndrome, or renal tubulopathy, were identified in two-thirds of the affected individuals.[13]

### Congenital Anomalies of the Kidney and Urinary Tract

The most common causes of chronic kidney disease (CKD) in children are congenital anomalies of kidney and urinary tract (CAKUT), followed by inherited kidney diseases such as polycystic kidney disease.[14] Both are very heterogeneous disorders with changes to a single gene, as well as complex, multigenic alterations.[14] NGS approaches allow the identification of disease-causing gene mutations in one single assay and therefore provide improved diagnostic possibilities.[7] To date, more than 20 monogenic CAKUT-causing genes have been identified. Whole genome linkage analysis can identify genomic regions of interest by comparing shared genes between affected family members. This is how single, rare, deleterious variants leading to CAKUT have previously been detected.[15,16]

### Autosomal Dominant Polycystic Kidney Diseases

A study by Trujillano et al.[17] demonstrated that using NGS to verify *PKD1* and *PKD2* mutations is superior, faster, and cheaper than the methods used routinely in the diagnosis of autosomal dominant polycystic kidney disease (ADPKD), such as Sanger sequencing.[18] ADPKD can also

manifest in early childhood; which is phenotypically similar to other ciliopathies, such as NPHP. In these situations, NGS helps to set the precise diagnosis, thereby improving prenatal counselling and patient management. Patients with ADPKD have an increased prevalence of intracerebral aneurysms, especially when additional risk factors are present. Therefore, they require follow-up.[19]

### Other Inherited Kidney Diseases

NPHP, chronic tubulointerstitial nephritis, is an autosomal recessive ciliopathy and the most common inherited cause of CKD in children.[7] The classic NPHP mutation is only detected in 30–40% of cases. NGS has led to the identification of other causal gene mutations such as the *SDCCAG8* mutation, which was identified by sequencing of 800 candidate genes in 10 families.[20] Accurate diagnosis of NPHP is important due to its initial unspecific clinical manifestation in children, and because of extrarenal manifestations such as neurological anomalies, hepatobiliary disease, or retinal degeneration.[19]

The power of NGS has also been demonstrated in a study that showed that patients with Alport syndrome who exhibit mutations of *COL4A3* and *COL4A4* genes, develop nephrotic-range proteinuria with histologic findings of focal segmental glomerulosclerosis (FSGS). Importantly, these patients did not suffer from graft loss after transplantation as could have been expected from patients with FSGS.[21] The diagnosis of Alport syndrome, as well as rarer diseases such as Sensebrenner's or Joubert's syndrome, has been tremendously accelerated through the widespread use of NGS. Diagnosis is now available in weeks in comparison to the months required by Sanger sequencing.[22,23]

## THE ROLE OF NEXT GENERATION SEQUENCING IN GLOMERULOPATHIES

NGS has led to the detection of genetic causes in diseases that had previously been regarded as essentially non-genetic. FSGS and IgA nephropathies are diseases in which mutational analysis has crucially influenced the understanding of the pathological mechanisms and their implications for clinical management.

### Steroid Resistant Nephrotic Syndrome and Focal Segmental Glomerulosclerosis

Nephrotic syndrome in children is classified into steroid resistant nephrotic syndrome (SRNS) and steroid sensitive nephrotic syndrome (SSNS). There is no causal treatment for SRNS, which inevitably leads to end-stage renal disease (ESRD).[24] SRNS is thus one of the main causes of ESRD in children and adolescents.[25] FSGS is a syndrome defined by specific glomerular damage and scarring. SRNS is a cornerstone of FSGS and often the underlying histopathological syndrome of SRNS in children.[26,27]

Exome sequencing of around 1,700 international families with SRNS revealed a single-gene cause in one-third of the patients. The earlier the patients developed nephrotic syndrome, the more often single gene causes were identified. In 1% of the patients, a mutation within the genes influencing the coenzyme Q10 pathway was detected. This might be a new biomarker for classification, but also a new target for treatment of SRNS.[25] Amongst children with sporadic SRNS, heterogeneous genetic alterations were as frequent as 58%, but were not identified in children with steroid sensitive disease.[28] Recently, a more time and cost efficient NGS technique was developed.[29] The technique allows for mutation analysis of 21 genes associated with SRNS. Eighty-one adult subjects with primary FSGS/SRNS were also investigated by Gast et al.[30] with a targeted NGS panel. They found that mutations within various collagen IV genes were the most frequent mutations detected. SSNS on the other hand, can also be genetically influenced, illustrated by *EMP2* mutations associated with familial SSNS.[24]

Genetic testing is also important when estimating the risk of recurrence of FSGS after transplantation. For example, none of the patients with mutations of *NPHS2* had a recurrence of FSGS after transplantation.[31] In this context, living donors can also be tested for silent mutations, which would increase the risk of recurrence.[27]

### IgA Nephropathy

The most common glomerulonephritis is IgA nephropathy, which leads to ESRD in 15–40% of cases. The epidemiology of IgA nephropathy is very heterogeneous. It is frequent within the Asian population, but rare in Africa. This suggests a genetic cause or predisposition.[32] A study examined the expression profiles of IgA nephropathy, via

transcriptome sequencing of microRNA (miRNA) with NGS techniques. Results showed that 85 miRNAs were differentially expressed and either up or downregulated in IgA nephropathy.[33] Mitochondrial DNA (mtDNA) was sequenced from the blood of patients with IgA nephropathy prior to kidney transplantation. It was found that patients with ESRD due to IgA nephropathy had more variations in mtDNA than healthy patients.[34]

### Membranous Nephropathy

miRNA was isolated and sequenced from peripheral blood lymphocytes of patients with membranous nephropathy (MN).[35] It was shown that miRNA profiles from patients with MN differ from healthy patients; more dysregulated miRNA could be found in patients with MN. Interestingly, more downregulated than upregulated miRNA were apparent in patients with MN, compared with the controls. miRNA could therefore play a role in the pathogenesis of MN, and could be used both as a diagnostic tool and as a potential therapeutic target.[34]

### Diabetic Nephropathy

Diabetic nephropathy (DN) is a main cause of ESRD in adults and is on the rise due to the increasing prevalence of diabetes worldwide,[36,37] although only 40% of all patients with Type 2 diabetes develop diabetic nephropathy. There is a known genetic and hereditary disposition for increased susceptibility to developing diabetic nephropathy, which has been investigated by NGS.[36] NGS might also yield novel therapeutic approaches to DN: transforming growth factor beta-1 (TGF-β1) influences the development and progression of diabetic nephropathy through glomerulosclerosis and tubular interstitial fibrosis.[37] A study sequenced the transcriptome of tubular cells stimulated with TGFβ1, which lead to insights into activated pathways such as NFκB. The following sequencing of kidney biopsies with DN revealed that patients shared 179 regulated genes with the *in vitro* tubular cell line. TGFβ1 might therefore be a candidate gene to target.[37]

## THE ROLE OF NEXT GENERATION SEQUENCING IN KIDNEY TRANSPLANTATION

NGS plays a role within the assessment before, and the monitoring and diagnosis of complications after kidney transplantation.

### Human Leukocyte Antigen Typing

In recent years, an abundance of new human leukocyte antigen (HLA) variants have been published. This has rendered HLA typing increasingly challenging, costly, and time consuming. NGS has increased sensitivity and facilitated the assessment of HLA status. HLA typing with NGS was compared with Sanger sequencing on a large scale in 2014. NGS was superior in time and cost efficiency. Results were concordant between both techniques.[38,39]

### Cell Mediated Rejection

T-cell mediated graft rejection (TCMR) is due to donor T cell responses towards major histocompatibility complexes. The mechanisms of TCMR were investigated through sequencing of the β locus of T cell receptors (TCRβ) in patients who underwent a combined kidney and bone marrow transplant. These patients commonly display immune tolerance without immunosuppressive therapy. TCRβ was sequenced using NGS prior to transplantation in order to monitor them during the adaptation towards tolerance. Results showed that donor reactive T cells were diminished in tolerant patients after transplantation. Characterisations of T cells with NGS prior to transplantation might therefore provide a new biomarker, improving the prediction of transplant outcome.[40] miRNA-10b is significantly downregulated in patients with rejected kidney allografts. A study showed that human renal glomerular endothelial cells transfected with miRNA-10b inhibitors showed increased apoptosis, proinflammatory cytokine release, and other cellular aspects also seen in graft rejection. These findings led the authors to suggest that miRNA-10b could be a novel therapeutic target in cell mediated graft rejection.[41]

### Monitoring of Rejection

Snyder et al.[42] described the potential use of NGS to non-invasively monitor the condition of the transplanted organ via cell-free DNA (cfDNA). Whole genome sequencing of the recipient was performed using NGS prior to the transplantation. By focussing on single nucleotide polymorphisms (SNPs), a unique fingerprint of the recipient's cfDNA was identified. After transplantation, donor cfDNA was sequenced and distinguished from host DNA through comparison in SNPs. Any increase in donor cfDNA was associated with 'injury'. In the case of heart transplant, an increase in donor

cfDNA was highly associated with acute rejection and even preceded the clinical event. This diagnostic and predictive approach could also have implications for kidney transplantation.[42]

### Diagnosis of Infections and Other Complications After Transplantation

Viral infections play an important part in the morbidity and mortality of patients after kidney transplantation.[43] Viral specific T cells can be detected through sequencing. NGS is of invaluable benefit in this case, because the T cell receptor has potentially indefinite variations according to the respective antigen immunisation.[44] A study showed that post-transplantational characterisation of T cells from urine, blood, and allograft with NGS helped to distinguish differential diagnosis such as infections as a reason for post-transplantation complications.[44] NGS can also be used to identify novel infectious pathogens. This was demonstrated by the discovery of a new arenavirus through the sequencing tissues originating from kidney transplant autopsies. This could not be achieved using routine diagnostics, such as polymerase chain reaction, microarray, or serological assays.[45]

## ROLE OF NEXT GENERATION SEQUENCING IN EPIGENETICS

Recent studies report that epigenetic variations contribute to individual patient susceptibility to develop progressive CKD.[46] Epigenetic research has advanced greatly with the emergence of NGS, which has allowed the detection of epigenetic modifications.[47] Epigenetics reflects functionally relevant changes to the genome without changing the underlying DNA sequence. Examples of such epigenetic changes are DNA methylation, histone modifications, but also long non-coding RNA fragments.[48] Epigenetic modifications can influence transcriptional regulation and thus affect gene expression, a phenomenon that is described as molecular memory. Each cell type has its specific epigenome modulated by environmental influences.[46,48] This cell type specific epigenome is defined during development and differentiation and preserved during cell division.[49] Environmental conditions, such as nutrition or smoking, can cause changes in the epigenome.[47]

Exploration of epigenetic modification using NGS can lead to new insights into the pathological mechanisms of kidney diseases. Altered DNA methylation for example, often activates or silences genes, and therefore alters gene expression in the kidney and consequently the rate of renal disease progression. The combination of chromatin immunoprecipitation assays with NGS is a strong method for identifying genome-wide DNA binding sites for transcription factors and other proteins. This can be used in epigenetic studies, e.g. for altered binding of transcription factors and histone modifications.[50,51]

### Epigenetics in Kidney Diseases

The epigenome is a factor in the development of complex gene-environmental diseases, including CKD.[49] Epigenetic dysregulation is supposed to add to cardiovascular morbidity.[52] As an example, miRNAs that can be identified via NGS represent epigenetic regulators influencing progression of cardiovascular disease.[52] Genetic variants and epigenetic changes in chromatin affect gene transcription in response to environmental stimuli. This is important in the regulation of diabetes development, including vascular changes, and can be analysed by NGS.[53] Recently, there has been progress regarding the definition of the contribution of epigenetics to the progression of fibrosis. Fibrosis is the common pathway of progressive CKD, but displays a huge variance in individual progression.[45] Recent studies of a genome-wide methylation screen show that epigenetic modifications lead to perpetuated fibroblast activation and fibrogenesis in the kidney.[54]

## CONCLUSION

NGS of the whole genome, exome, or epigenome plays a crucial role for today's generation of nephrologists, both in basic and experimental research, but also in the clinical setting. Diagnosis and classification of kidney diseases have improved, but also therapeutic decision-making, which helps to avoid unnecessary therapies.

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# THE ROLE OF GENDER IN CHRONIC KIDNEY DISEASE

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 09.02.16 **Accepted:** 16.03.16
**Citation:** EMJ. 2016;1[2]:58-64.

## ABSTRACT

Chronic kidney disease (CKD) is a common disease worldwide and is associated with high rates of morbidity and mortality. This review discusses several aspects of the relationship between gender and CKD. While the prevalence of CKD tends to be higher in women, the disease is more severe in men, who also have a higher prevalence of end-stage renal disease. Most of the evidence in the current literature suggests a higher progression rate and mortality risk of CKD in men compared with women, except in post-menopausal women and diabetic patients. However, the decrease in glomerular filtration rate and the increase in the level of albuminuria are more prominent mortality risk factors among women. Sex hormones are thought to play a major role in the biological mechanisms associated with variability in CKD prevalence and characteristics between men and women. Animal studies have demonstrated the harmful influence of testosterone and protective influence of oestrogen on several biological processes that are involved in kidney injury. However, the role of sex hormones in explaining gender-related differences in CKD in humans has not yet been established. In summary, gender has an important influence on several aspects of CKD. Further research is needed to find additional gender-related characteristics in CKD and to identify the mechanisms of sexual dimorphism in CKD.

Keywords: Renal failure, glomerular filtration rate (GFR), sex hormones, end-stage renal disease (ESRD).

## INTRODUCTION

Chronic kidney disease (CKD), defined by reduced estimated glomerular function rate (eGFR) and/or albuminuria levels, is a worldwide health problem due to the significant rate of morbidity and mortality associated with it. CKD is associated with an increased risk of all-cause mortality and cardiovascular mortality, and progression to end-stage renal disease (ESRD).[1] To establish better prevention strategies and enable early detection, much effort has been made to identify risk factors associated with CKD development. In this respect, various studies have been conducted to assess the effect of gender on the prevalence, progression, and characteristics of CKD. In this review, the main aspects of gender influence on CKD are discussed.

When addressing the difference between CKD in men and women, we must take note of the fact that eGFR (commonly used in studies) is based on a patient's sex, among other variables. The two most common equations for assessing eGFR, the MDRD (Modification of Diet in Renal Disease) and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equations, both use sex as a variable. They are based on the assumption that for a given creatinine level, men will have higher levels of kidney function than women due to higher muscle mass and increased creatinine generation among men.[2] Thus, in the lack of studies using the gold standard measurement of glomerular filtration rate (GFR) (which is the measurement of inulin), results regarding gender influence on CKD are often biased due to the use of sex dependent equations.

## PREVALENCE OF RENAL DISEASE AND END-STAGE RENAL DISEASE AMONG MEN AND WOMEN

Based on the most recent US Renal Data System (USRDS) annual data report,[3] the prevalence of chronic renal failure between the years 2007 and

2012 was higher in women (15.1%) than in men (12.1%). Women had a higher prevalence of high urinary albumin to creatinine ratio (9.6% versus 8.1% in men) and a higher prevalence of decreased GFR (7.6% versus 5.4% in men; decreased GFR is defined as eGFR <60 mL/min/1.73 $m^2$). It is important to mention that several previous studies have shown opposing data regarding this issue.[3-8] For example, a French epidemiologic study showed a higher incidence rate of chronic renal failure in men,[4] whereas a Chinese cross-sectional study[5] demonstrated similar CKD prevalence among men and women. There might be a geographic variability in the effect of gender on the prevalence of CKD, as shown in Table 1. Nonetheless, the incidence of ESRD appears to be higher in men than women. Based on the most recent US Renal Data System data,[3] 57.8% of the patients with a new onset ESRD were men. Furthermore, 56.3% of the prevalent dialysis patients were males, as were 59.7% of the kidney transplant recipients in the USA. Several other studies[9-11] found similar results, as shown in Table 2.

It should be noted that a natural decline in GFR with age is present in the healthy population.[12]

**Table 1: Geographic variability in gender-associated prevalence of CKD.**

| Study | Year of publication | GFR assessment method | Percentage of women with CKD | Percentage of men with CKD | Sample size |
|---|---|---|---|---|---|
| Swedish National Study on Ageing and Care[6] | 2014 | Cystatin C | 23.2 | 17.7 | 1,252 |
| Swedish National Study on Ageing and Care[6] | 2014 | Creatinine | 23.08 | 15.01 | 1,252 |
| Ile de France district (France)[4]* | 1996 | Direct level of creatinine | 0.0179 | 0.0348 | 10,660,000 |
| USA NHANES population[3] | 2007-2012 | Lower estimated GFR and high urinary albumin creatinine ratio | 15.1 | 12.1 | No Data |
| Framingham (Massachusetts, USA)[7] | 1999 | Creatinine level (gender-specific cut-off) | 8 | 8.7 | 6,233 |
| Beijing (China)[5] | 2008 | Creatinine (using the MDRD equation), albuminuria | 12.7 | 13.3 | 13,925 |
| Middle and old-aged population of Beijing (China)[8] | 2005 | Creatinine (using the MDRD equation) | 12.6 | 13.2 | 2,310 |

*The reported data is the annual incidence of CKD.
CKD: chronic kidney disease; GFR: glomerular filtration rate; MDRD: modification of diet in renal disease.

**Table 2: Geographic variability in gender-associated cumulative incidence of ESRD.**

| Study | Year of publication | Percentage of women with ESRD | Percentage of men with ESRD | Sample size |
|---|---|---|---|---|
| Predictors of ESRD in Finland[9] | 2010 | 0.27 | 0.46 | 25,821 |
| Washington Country, Maryland[10] | 2003 | 0.49 | 0.78 | 23,534 |
| Japan[11]* | 1999–2000 | 0.0189 | 0.032 | No Data |
| USA white people[75] | 2006 | 0.0139 | 0.0169 | No Data |
| Norway[75] | 2006 | 0.0036 | 0.007 | No Data |

*The reported data is the incidence of CKD (but not cumulative incidence).
ESRD: end-stage renal disease.

In a study which used the gold standard method for assessing GFR, a significant decrease in GFR with age among men, but not among women, was observed.[13] Furthermore, research from India showed a significantly higher mean eGFR among women aged 41–45 years, yet no significant difference between men and women in the 20–30 or 30–40 year age groups.[14] In contrast, in an Israeli longitudinal study[12] that assessed the natural rate of decline in renal function with age, no significant effect of gender on the natural decline of GFR with age was observed.

## THE INFLUENCE OF GENDER ON DISEASE PROGRESSION

Though the prevalence of chronic renal failure may be higher in women, the incidence of ESRD seems to be higher in men, indicating that the progression rate of renal disease may be faster in men than women. Though the literature regarding this issue is conflicting and inconclusive, most studies support this assumption.

In a large meta-analysis published in 2000,[15] a significant correlation between male gender and disease progression of IgA nephropathy, membranous nephropathy, autosomal dominant polycystic disease, and 'chronic renal disease of unknown aetiology' was demonstrated. In addition, a Swedish cohort study[16] found that the male sex is associated with a greater risk of future need of renal replacement therapy among patients with chronic renal failure, in comparison with the female sex. In another study,[17] which examined the variation in GFR among patients with CKD Stage 3, a more rapid decline in GFR in men was demonstrated. Similar findings were observed in a large cohort of patients with CKD Stage 4.[18]

A smaller study performed on participants of the MDRD study[19] indicated a slower mean GFR decline in women compared with men, particularly among younger women. However, the association between gender and the rate of GFR decline became non-significant after adjusting for differences in blood pressure, proteinuria, and high-density lipoprotein (HDL) cholesterol. In this study, women had different renal diagnoses, less proteinuria, and lower serum creatinine levels for a given GFR compared with men. Conflicting results were found in a large meta-analysis performed on a database of patients enrolled in a randomised clinical trial for evaluating the efficacy of ACE (angiotensin-converting-enzyme) inhibitors in slowing renal disease progression,[20] in which a similar rate of progression in men and women was observed. Further, adjustment for other factors which affect the rate of renal progression suggested that progression may be faster in women. However, most of the female participants were of post-menopausal age, and therefore these findings may not extend to younger women. In a large cohort of 24,682 patients aged ≥50 years,[21] female sex was a risk factor for developing ESRD (hazard ratio: 1.48); most of the women in this study were also of post-menopausal age. The authors suggest that as the prevalence of cardiovascular mortality increases in both men and CKD patients, mortality in men might be higher occurring before the development of ESRD. A large meta-analysis performed on a global consortium[22] did not show a gender-related difference of eGFR with the risk of developing ESRD. For specific values of urinary albumin creatinine ratio, women showed a slightly higher risk of developing ESRD, compared with men. Results were obtained from a pooled analysis of 13 CKD cohorts containing over 38,000 participants.

The effect of gender on diabetic renal disease is much more debatable. Whilst a number of studies show that male gender is a risk factor for diabetic kidney disease (DKD),[23-25] others demonstrate that women are at higher risk of developing the disorder,[26,27] and some found no significant difference between men and women in terms of risk.[28,29] Recent research, which was designed to assess the influence of gender on the incidence of CKD among diabetic patients, concluded that diabetic women had a significantly higher incidence of CKD.[30] Furthermore, diabetic women had a higher (but not significant) incidence of microalbuminuria. This study included both Type 1 and Type 2 diabetic patients. In another study, which was conducted using the Pathways Study database, women had a similar prevalence of DKD in comparison with men, but had an increased prevalence of advanced DKD.[31]

In conclusion, current evidence regarding the effect of gender on DKD is contradictory, though it seems that a protective role of female sex on the kidney is lost in the case of DKD. One reason for this finding might be an imbalance in sex hormones associated with diabetes.[32]

## GENDER ASPECTS REGARDING MORTALITY

Several studies have shown a reduced risk of mortality among women with CKD, compared with men.[17,33,34] Among CKD patients, the most common causes of death were cardiovascular disease, cancer, and infections.[34] In a large meta-analysis performed on a global consortium of over 2 million participants,[22] risks of all-cause mortality and cardiovascular-related mortality were higher among men for all levels of eGFR and for all levels of albuminuria. However, among participants with lower values of eGFR and participants with higher levels of albuminuria, the elevation in mortality risk was steeper for women. Thus, changes in GFR and albuminuria levels seem to be more significant risk factors for mortality (all-cause and cardiovascular-related mortality) in women. The similarity in the risk of developing ESRD for a given eGFR and level of albuminuria in both sexes leads us to speculate that the stronger correlation found between renal function and mortality among women was not due to renal disease-related mortality. It might reflect a higher risk of non-kidney disease mortality. Other specific risk factors might contribute to the higher risk of cardiovascular mortality among women with low GFR. These include hypertension, serum glucose level, serum lipids (total cholesterol level, low-density lipoprotein [LDL], triglycerides), and more. Further prospective studies are needed for evaluating these risk factors in large cohorts.

## THE INFLUENCE OF GENDER ON OTHER RISK FACTORS FOR DEVELOPING CHRONIC KIDNEY DISEASE

Several risk factors for developing CKD among healthy people have been defined. Hypertension, diabetes, and cardiovascular disease are considered the most important risk factors for CKD. Other considerable risk factors for CKD are hyperlipidaemia, obesity, metabolic syndrome, and smoking.[35] Elevated levels of C-reactive protein[36] and of homocysteine[37] were both found to be independent risk factors for the development of CKD.

The effect of gender on these risk factors has also been examined. Several studies have shown that obesity (high body mass index) is a risk factor for developing CKD among women but not among men.[38,39] Other studies have yielded the opposite results.[40,41] Lately, it has been shown that the correlation between homocysteine and CKD is similar in men and women, in contrast to the results of a previous Japanese study in which elevated homocysteine was a risk factor for CKD only in women.[42] An Italian study[43] demonstrated gender differences regarding recognised risk factors for CKD: men had a higher prevalence of overweightness, having a higher waist circumference, and a higher blood pressure, whereas women had a higher prevalence of haematuria and leukocyturia, which are early manifestations of CKD.

## PROTECTIVE AND PATHOGENIC MECHANISMS

Many possible mechanisms for the protective effect of female gender on CKD patients have been suggested. These include gender differences in kidney anatomy, kidney haemodynamic stress response, effect of sex hormones, diet, lipid metabolism, and blood pressure.[44,45] Anatomically, the kidney is usually larger in men, due to a larger body surface area. Some studies have shown a smaller number of glomeruli in female kidneys.[44] The haemodynamic stress response of the kidney differs between men and women; men may develop higher filtration fraction in response to angiotensin II infusion.[46] During hyperglycaemia, women exhibited a reduction in renal blood flow and an increase in renal vascular resistance and filtration fraction, whereas males exhibited no significant renal haemodynamic changes.[47] These findings may explain the lack of renal protection among diabetic women.

Lifestyle differences between men and women has been suggested as another possible explanation for the influence of gender on CKD. A high protein and high caloric dietary intake, which characterises men more than women, is associated with the development and the progression of kidney disease. High levels of LDL, triglycerides, and uric acid, and low levels of HDL are associated with accelerated kidney disease progression.[48] These trends are more common in men, and are influenced by nutrition and lifestyle.

The role of sex hormones in the pathogenesis of renal injury has gained a lot of attention. Several animal studies demonstrate a harmful influence of testosterone and protective influence of oestrogen on processes involved in kidney injury.[49] Testosterone induces podocyte apoptosis

(playing an important role in the development of glomerulosclerosis),[50] and TGF-β1 expression[51] (which is connected to tissue fibrosis), while oestradiol inhibits these processes.[52,53] It was demonstrated that testosterone induces proximal tubular cell apoptosis in human cells *in vitro*.[54] In addition, oestradiol has a direct influence on mesangial cells, decreasing extracellular matrix production and glomerulosclerosis.[44]

Nitric oxide (NO) synthase activity is also influenced by sex hormones. For example, oestrogen depletion has been associated with a decrease in the level of NO synthesis (endothelial and inducible NO synthase) in the kidney medulla.[55] Another study found an age-dependent reduction in NO synthase activity in the kidney cortex of male rats,[56] but not in female rats. Generally, NO synthase inhibition is associated with renal injury.[57] Recent animal studies have shown a protective role of NO in acute kidney injury.[58,59] However, there is some evidence of malicious influence of NO on kidney disease.[60] Thus, the role of NO in renal injury is complex as it varies depending on cell type and NO isoform. Additional influences of sex hormones on key factors in kidney injury have been noted: the renin–angiotensin system is induced by testosterone and inhibited by oestrogen; oestradiol inhibits the synthesis of endothelin, as well as its vasoconstrictor and inflammatory effects;[44] oestrogen also plays a role in decreasing kidney oxidative stress by suppressing NADPH oxidase activity.[61]

To summarise, various animal studies have shown that testosterone is associated with a rapid progression of kidney injury by several mechanisms. This could explain the faster progression rate of CKD among men compared with women. However, extrapolation of these findings to the human kidney is problematic. Testosterone levels in men, for example, decrease as kidney disease worsens.[62,63] Advanced CKD is associated with lower levels of testosterone, prolactin, and anti-Müllerian hormone,[64] as well as higher levels of gonadotropin. Some evidence suggests that testosterone has a protective role on the kidneys.[65] Androgen deprivation therapy, a treatment for advanced prostate cancer, is associated with an increased risk of acute kidney injury.[66] In a pig model, testosterone induced renal blood flow by enhancing NO production.[67] It has been shown that low testosterone levels in CKD in men can predict mortality.[68] Testosterone deficiency is associated with several cardiovascular risk factors, including metabolic syndrome, diabetes, hypertension, obesity, and atherosclerosis.[69] Thus, the decrease in levels of male androgens among CKD patients might play a role in the higher cardiovascular mortality rate among men with CKD.[63]

As mentioned previously, according to many animal studies, oestrogen might have a protective role on the kidney. In humans, the higher risk of CKD progression among post-menopausal women (concluded from the fact that studies using post-menopausal women did not show a higher progression rate of CKD in men)[20,21] supports the protective role of oestrogen. We would expect that hormonal therapy (either hormone replacement therapy in post-menopausal women or contraceptive use in pre-menopausal women) would improve kidney function. Clinical studies that examined this assumption yielded opposing results. Several demonstrated that post-menopausal hormonal replacement therapy was associated with a lower risk of albuminuria and a higher creatinine clearance.[70,71] In contrast, one study[72] showed a dose related association of post-menopausal oestrogen replacement therapy with loss of kidney function, whilst another[73] found that oral contraceptives are associated with a development of macroalbuminuria among Type 1 diabetic patients. Furthermore, a case report study performed on historical data found that the use of oral contraceptives and hormone replacement therapy is associated with increased risk of microalbuminuria.[74]

## CONCLUSIONS

Despite conflicting data regarding the influence of gender on CKD, some conclusions can be made. The prevalence of CKD tends to be higher in women, whereas the disease in men is more severe. Much of the evidence in the current literature indicates a higher progression rate of CKD in men, with the exception of post-menopausal women and diabetic patients. Mortality rate in patients with CKD is higher among men. The findings of Nitsch et al.[22] demonstrate that as kidney disease progresses in women, the elevation in mortality risk increases. This effect has not been shown in men, and further research is needed to understand the role of gender in the association between renal disease severity and mortality.

It seems that the influence of sex hormones on several biological processes involved in kidney injury plays a major role in creating gender differences in CKD; many animal and *in vitro* studies support this assumption. However, the same role has not been demonstrated in human-based research, and numerous studies refute this theory. In our opinion, as the level of sex hormones is influenced by several variables (diseases, medications, age, etc.) in addition to gender itself, it is difficult to extrapolate the findings from animal studies to the human population. Further research is therefore needed in this area.

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# CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN NEVER-SMOKING WELDING WORKERS

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 17.12.15 **Accepted:** 16.03.16
**Citation:** EMJ. 2016;1[2]:65-70.

## ABSTRACT

Introduction: Results from several studies indicate that workplace exposure to welding fumes is associated with increased frequency of chronic obstructive pulmonary disease (COPD) in exposed workers.
Objective: To assess the prevalence and characteristics of COPD in never-smoking welders.
Methods: A cross-sectional study including 53 never-smoking male welders (aged 35–60 years) was performed, and an equal number of never-smoking male office workers were studied as a control. Evaluation of examined subjects consisted of the completion of a questionnaire, baseline spirometry, and bronchodilator reversibility testing.
Results: We found a higher prevalence of respiratory symptoms in welders, with significant differences in cough and phlegm. The majority of the chronic respiratory symptoms in welders were work-related. The mean values of all measured spirometric parameters registered with both pre and post-bronchodilator spirometry in welders were significantly lower than in office workers. The prevalence of COPD was significantly higher in welders than in office workers (15.1% versus 3.8%, p=0.041). COPD in both welders and office workers was similar in those aged <45 years.
Conclusion: Our findings support data about the relationship between workplace exposure to welding fumes and persistent airflow limitation.

Keywords: Airflow limitation, baseline spirometry, bronchodilator reversibility testing, questionnaire, welders.

## INTRODUCTION

Over recent decades, chronic obstructive pulmonary disease (COPD) has become an important public health problem at a global level. According to the projection of the Global Burden of Disease Study, COPD, which ranked as the sixth leading cause of death in 1990, will become the third leading cause of death worldwide by 2020.[1] Across the world, cigarette smoking is the most commonly encountered risk factor of COPD, with a clear dose-response relationship. Although cigarette smoking is a major risk factor of COPD, there is consistent evidence that a substantial proportion of COPD cases cannot be explained by smoking. Other noxious particles and gases, such as workplace dust, vapours, fumes or gases, indoor air pollution from burning biomass fuels (caused by cooking and heating), and urban outdoor air pollution are important risk factors of COPD. According to the available evidence, the contribution of workplace exposure to noxious particles and gases to the development of the disease is estimated as being 15–20% of all COPD cases; around 4,000 COPD deaths every year are related to workplace exposures, 40% of COPD patients are below retirement age, and a quarter of those below retirement age are unable to work at all.[2,3]

The development of COPD as a consequence of workplace exposure is a matter of growing interest, importance, and even controversy. There is no doubt that certain workplace exposures enhance

the risk of COPD, and may do so independently of, or in concert with cigarette smoking. The evidence is most coherent for work that entails exposure to coal, silica, welding fumes, cadmium fumes, cotton dust, farming dusts, grain dust, or wood dust. For other occupations the evidence is less conclusive and warrants further studies, particularly in roles that entail exposure to diesel fumes, diisocyanates, polycyclic hydrocarbons, asbestos, and iron/steel fumes.[4-6] This study aimed to assess the prevalence and characteristics of COPD in never-smoking male workers exposed to welding fumes.

## METHODS

### Study Design and Setting

The study compared respiratory symptoms in the last 12 months, spirometric findings, and prevalence of COPD between a group of welding workers and a group of office workers in a cross-sectional analysis. The study took place from September 2014–January 2015 at the Institute for Occupational Health of the Republic of Macedonia (IOH of RM), Skopje.

### Subjects

The examined group of welding workers included 53 males (age 35–60 years, duration of employment 14–27 years) working in stainless steel (SS) welding. They worked in a metallurgic plant in two work shifts, each lasting for a period of 8 hours. Their working tasks included welding and cutting SS, i.e. steel containing nickel and chromium, which was performed in a large working area (approximately 160 $m^2$) with a central ventilation system. The welding technique used is known as flux core arc welding, a welding method used for carbon steels, low alloy steels, and SS. In this welding process the consumable electrode (the welding rod) is continuously fed from a spool and an electric arc flows between the electrode and the base metal. This type of welding is characterised by the generation of a large amount of fumes, due to the high electrical currents and the flux-cored electrode. During the work shift, the welding workers used protective clothing, gloves, masks, and glasses. All examined welders were never-smokers, i.e. individuals who have never smoked at all, or have never been daily smokers and have smoked <100 cigarettes in their lifetime.[7,8] In addition, data from 53 never-smoking males employed as office workers, matched to the welders by age, served as controls. No individual from either group had been diagnosed with chronic respiratory disease by a physician (such as asthma, COPD, bronchiectasis, etc.), nor were they treated with bronchodilators and/or corticosteroids.

### Questionnaire

An interviewer-led questionnaire was completed by all study subjects. The questionnaire consisted of five parts: respiratory symptoms in the last 12 months and their work-relatedness, work history, passive smoking, accompanying diseases, and history of COPD or chronic bronchitis in first-degree relatives (parents and/or siblings). Overall and specific respiratory symptoms (cough, phlegm, dyspnoea, wheezing, and chest tightness) in the past 12 months were defined according to the European Community for Coal and Steel Questionnaire (ECCS-87), and the European Community Respiratory Health Survey (ECRHS) questionnaire.[9,10] The work-relatedness of the respiratory symptoms was defined as having worsened during or after the work shifts and their improvement in the periods away from work.[11] The work history was described through questions about current and previous jobs, work activities at the current workplace, characteristics of the exposure at the current workplace, and the use of protective equipment. Passive smoking or exposure to environmental tobacco smoke (ETS) was defined as an exposure to tobacco smoke from smoking by others, i.e. being in the presence of at least one smoker in the household and/or at the workplace.[12,13]

### Spirometric Measurements

The spirometric measurements included baseline (pre-bronchodilator) spirometry and bronchodilator reversibility testing (post-bronchodilator spirometry). They were performed using a Ganshorn SanoScope LF8 (Ganshorn Medizin Electronic GmbH, Germany). Forced vital capacity (FVC), forced expiratory volume in 1 second ($FEV_1$), $FEV_1$/FVC, and maximal expiratory flow (MEF) at 75%, 50%, 25%, and 25–75% of FVC ($MEF_{75}$, $MEF_{50}$, $MEF_{25}$, and $MEF_{25-75}$, respectively) were measured, recording the best result from three measurements of $FEV_1$, all of which were within 5% of each other. According to the recommendations of the European Respiratory Society (ERS) and the American Thoracic Society (ATS), the results of the spirometric measurements were expressed as percentages of the predicted values.[14,15]

**Table 1: Characteristics of the examined groups.**

| Variable | Welders (n=53) | Office workers (n=53) |
|---|---|---|
| Age (yrs)<br><45<br>>45 | 48.4±5.8<br>25 (47.2%)<br>28 (52.8%) | 49.2±5.1<br>24 (45.3%)<br>29 (54.7%) |
| Body mass index (kg/m$^2$)<br><25<br>>25 | 25.9±1.9<br>26 (49.2%)<br>27 (50.8%) | 26.2±1.7<br>25 (47.2%)<br>28 (52.8%) |
| Duration of employment at the workplace (yrs) | 21.8±3.9 | 20.5±4.3 |
| Duration of employment <20 yrs | 24 (45.3%) | 26 (49.2%) |
| Duration of employment >20 yrs | 29 (54.7%) | 27 (50.8%) |
| Family history of COPD or CB | 8 (15.1%) | 6 (11.3%) |
| Passive smoking | 21 (39.6%) | 18 (33.9%) |
| Accompanying diseases<br>Arterial hypertension<br>Diabetes mellitus Type 2<br>Peptic ulcer | <br>9 (16.9%)<br>5 (9.4%)<br>3 (5.7%) | <br>8 (15.1%)<br>6 (11.3%)<br>5 (9.4%) |

Numerical data are expressed as mean value with standard deviation; frequencies as number and percentage of study subjects with certain variables.
COPD: chronic obstructive pulmonary disease; CB: chronic bronchitis.

Post-bronchodilator spirometry, i.e. bronchial reversibility testing, was performed according to the Global initiative for chronic Obstructive Lung Disease (GOLD) spirometry guide.[14] Spirometric measurements were performed 20 minutes after the administration of 400 µg salbutamol by metered dose inhaler through a spacer and by comparing registered values with those registered by baseline (pre-bronchodilator) spirometry. Persistent airflow limitation was considered if the post-bronchodilator $FEV_1$/FVC remained <0.70, independent of the degree of $FEV_1$ reversibility. In addition, the degree of $FEV_1$ reversibility was calculated as a percentage of $FEV_1$ reversibility ([post-bronchodilator $FEV_1$ - pre-bronchodilator $FEV_1$]/pre-bronchodilator $FEV_1$ x 100).

## Chronic Obstructive Pulmonary Disease Diagnosis

According to the GOLD recommendations, COPD was considered when post-bronchodilator $FEV_1$/FVC was <0.70 in symptomatic subjects (dyspnoea, chronic cough, or sputum production) with a history of exposure to risk factors for the diseases (noxious particles and gases).

## Statistical Analysis

Statistical analysis was performed using SPSS v.20 (IBM, New York, USA) version 11.0 for Windows. Continuous variables were expressed as mean values with standard deviation and the nominal variables as numbers and percentages. Analyses of the data included testing the differences in prevalence, comparison of the means, testing the association between COPD and studied variables by chi-square test (or Fisher's exact test where appropriate), and independent samples t-test. A p-value <0.05 was considered as statistically significant.

# RESULTS

Demographic characteristics of the study subjects were similar in both examined groups (Table 1). While we found a higher prevalence of overall respiratory symptoms in welders than in office workers, the results were not statistically significant. Particular chronic respiratory symptoms were more prevalent in welders, with a statistically significant difference for cough and phlegm (Table 2). The majority of respiratory symptoms in the last 12 months in welders were related to their work (86.4%), with the highest reported for cough (73.3%) and phlegm (80.0%). Such work-related symptoms were reported by 16.7% of the office workers with respiratory symptoms.

The mean values of all measured spirometric parameters registered with baseline spirometry

were significantly lower in welders. The mean post-bronchodilator values of all spirometric parameters were also significantly lower in welders (Table 3). In addition, the mean $FEV_1$ reversibility (percentage of $FEV_1$ reversibility) was significantly higher in welding workers (8.7±2.3 versus 3.8±0.9; p=0.000; independent samples t-test).

Eight subjects among the welders and two subjects among the office workers met the criteria for diagnosis of COPD (15.1% versus 3.8%, p=0.041; Fisher's exact test). All welders with COPD reported that their symptoms were work related. According to the GOLD classification of COPD severity, all welders and office workers with established COPD can be classified as subjects with mild COPD ($FEV_1$/FVC <0.70; $FEV_1$ ≥80% predicted), i.e. as a GOLD 1.[16]

All COPD cases in both examined groups were aged >45 years. The duration of employment at the workplace in six welders with COPD (87.5%) was >20 years. In addition, in 1 of the 2 office workers with COPD, duration of employment at the workplace was registered as >20 years. There was no significant association between COPD and other variables (body mass index, family history for COPD or chronic bronchitis, and passive smoking) in subjects with COPD from both examined groups.

## DISCUSSION

There is strong evidence that COPD due to occupational exposures has markedly increased during the last few decades, becoming an important cause of morbidity and mortality in many occupations. Workplace agents considered as risk factors for COPD include: mineral dusts (coal, silica, silicates, oil mist, and man-made fibres), organic dusts (cotton, grain, wood, and paper dust), metals (welding fumes, cadmium, and vanadium), gases (sulfur dioxide and ammonia), and smoke (internal combustion engine exhaust), as well as mixed exposure, poorly defined as industrial vapours, gases, dusts, and fumes.[17,18] This study assessed the impact of specific occupational exposure in welding workers on the development of COPD. A control group consisted of an equal number of male office workers, matched to welders by age and smoking status.

As we aimed to exclude the effect of smoking on the COPD development, we examined only never-smoking workers to avoid use of regression analyses. The examined groups included subjects with similar demographic characteristics. More than one-third of the subjects in both examined groups were exposed to ETS; this is similar to the prevalence of passive smoking among workers in the Republic of Macedonia documented in our previous studies.[19,20]

Welding fumes are a complex mixture of metallic oxides (chromium, nickel, iron, copper, etc.), silicates, and fluorides. Fumes are formed when a metal is heated above its boiling point and its vapours condense into very fine particles. The composition of welding fumes can be changed by the vapours and fumes that come from coatings and residues on the metal being welded (cadmium plating, lead oxide primer paints, zinc on galvanised steel, plastic coatings, vapours from paints and solvents, etc.).[21]

**Table 2: Prevalence of overall and specific respiratory symptoms in the last 12 months.**

| Respiratory symptoms in the last 12 months | Welders (n=53) | Office workers (n=53) | P-value* |
|---|---|---|---|
| Overall respiratory symptoms | 22 (41.5%) | 12 (22.6%) | 0.067 |
| Cough | 15 (28.3%) | 5 (9.4%) | 0.013 |
| Phlegm | 8 (15.1%) | 2 (3.8%) | 0.046 |
| Dyspnoea | 6 (11.3%) | 4 (7.5%) | 0.506 |
| Wheezing | 5 (9.4%) | 3 (5.7%) | 0.462 |
| Chest tightness | 5 (9.4%) | 5 (9.4%) | 1.000 |

Data are expressed as number and percentage of study subjects with certain variables.
*Tested by chi-square test (or Fisher's exact test where appropriate).

**Table 3: Mean baseline and post-bronchodilator values of spirometric parameters.**

| | Baseline spirometry | | | Post-bronchodilator spirometry | | |
|---|---|---|---|---|---|---|
| Spirometric parameter | Welders (n=53) | Office workers (n=53) | P-value* | Welders (n=53) | Office workers (n=53) | P-value* |
| FVC (%pred) | 91.7±11.2 | 95.9±9.8 | 0.042 | 92.9±11.4 | 98.3±10.1 | 0.011 |
| $FEV_1$ (%pred) | 75.7±7.8 | 84.3±11.3 | 0.011 | 78.9±12.8 | 87.6±10.7 | 0.009 |
| $FEV_1$/FVC | 0.76±0.05 | 0.83±0.03 | 0.008 | 0.76±0.04 | 0.84±0.04 | 0.003 |
| $MEF_{75}$ (%pred) | 65.4±10.7 | 76.4±12.8 | 0.001 | 67.8±13.1 | 78.1±8.3 | 0.000 |
| $MEF_{50}$ (%pred) | 60.2±12.1 | 70.3±13.1 | 0.000 | 63.1±13.9 | 71.7±10.7 | 0.000 |
| $MEF_{25}$ (%pred) | 53.2±12.8 | 64.1±11.2 | 0.000 | 54.9±12.4 | 66.7±13.1 | 0.000 |
| $MEF_{25-75}$ (%pred) | 62.3±13.9 | 76.7±14.1 | 0.000 | 66.8±14.7 | 78.7±12.8 | 0.000 |

Data are expressed as mean value of certain spirometric parameter with standard deviation.
*Tested by independent-samples t-test.
FVC: forced vital capacity; $FEV_1$: forced expiratory volume in one second; $MEF_{75}$, $MEF_{50}$, $MEF_{25}$, $MEF_{25-75}$: maximal expiratory flow at 75%, 50%, 25%, and 25-75% of FVC, respectively; %pred: percentage of predicted value.

The prevalence of overall respiratory symptoms in the welders was higher than in the office workers. Prevalence of specific respiratory symptoms was also higher in the welders, with a statistically significant difference for cough and phlegm. The majority of respiratory symptoms in the welders were work-related, reaching >70% for cough and phlegm.

The results from spirometric measurements demonstrated significantly lower mean values of all measured spirometric parameters in welders than in office workers. These findings were registered by both baseline and post-bronchodilator spirometry. In addition, we found significantly higher mean $FEV_1$ reversibility in welders than in office workers. The effect of workplace exposure to welding fumes on the lung function of exposed workers has been documented in several studies. In a cross-sectional analysis of 106 subjects working as welders for 4–34 years and 80 matched controls, Bogadi-Sare[22] found significantly lower $FEV_1$/FVC values in exposed workers, independent of their smoking status. In addition, in a study that included 657 shipyard workers exposed to welding fumes, Gennaro et al.[23] found a significant impairment of lung function in the workers with a duration of exposure >20 years as compared with lung function of the workers with shorter workplace exposure. The higher mean $FEV_1$ reversibility in welders compared with the office workers registered in the present study may be due to the higher degree of airflow inflammation in workers exposed to welding fumes than in the unexposed controls.

COPD prevalence in never-smoking subjects working in dusty occupations obtained from the studies performed at the IOH of RM varied between 10.8% in grain workers, 11.4% in cotton workers, and 14.9% in bricklayers. At the same time, COPD prevalence in the office workers studied as a control varied from 2.3–4.3%.[24-26] Despite controls in these and the present study, never-smokers with no occupational exposure to noxious particles and gases, the influence of other COPD risk factors (e.g. genetic factors, childhood respiratory infections, household exposure to ETS, etc.) could not be excluded. In the present study, COPD prevalence in welders was 15.1%; around 3-fold higher than its prevalence in matched office workers (3.8%). COPD in both welders and office workers was similar in those aged <45 years. Similar results (i.e. COPD prevalence of around 15%) were registered by Koh et al.[27] in a study including a group of male welders working at two shipyards (mean age 48 years, mean duration of exposure 15 years, and mean cumulative exposure 7.7 mg/m$^3$). Odds ratios for COPD were significantly higher in the middle and high exposure groups compared with the low fume exposure group.[27]

The present study must be interpreted within the context of its limitations: a relatively small

number of the subjects in the study groups could have certain implications on the data obtained and its interpretation; the impact of the healthy workers' effect on the data obtained should also not be excluded (the healthy workers' effect is recognised as the most common selection bias in occupational studies, and it may partially or completely mask excess mortality and morbidity caused by harmful workplace exposure); and finally, environmental measurements were not performed, so the effects of the type and the level of exposure to welding fumes on the examined variables could not be documented. The strength of the study is its extensive examination of respiratory effects of workplace exposure associated with a certain welding technique on never-smoking workers.

## CONCLUSION

In conclusion, this cross-sectional study found a higher prevalence of respiratory symptoms in the last 12 months, significantly higher mean values of spirometric parameters, and significantly higher prevalence of COPD in never-smoking welding workers, than in matched office workers. COPD in welding workers was closely related to age and duration of employment at the workplace. Our results confirm the need for constant improvement of preventive measures, i.e. proper engineering control and respiratory protective equipment, as well as regular periodical medical examinations of exposed workers, in order to protect their health from the risks of welding exposure.

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# THE RISE AND FALL OF ROUTINE MANUAL THROMBECTOMY FOR ST-ELEVATION MYOCARDIAL INFARCTION

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 26.01.16 **Accepted:** 03.03.16
**Citation:** EMJ. 2016;1[2]:71-75.

## ABSTRACT

Manual thrombectomy (MT) with an aspiration catheter is frequently used in primary percutaneous coronary intervention (PPCI) for acute myocardial infarction with ST-segment elevation (STEMI). It is used to reduce the thrombus burden and the risk of no-reflow in the infarct related artery. This article summarises a chronological overview of the available evidence for its routine use in PPCI. An early series of small randomised controlled trials (RCTs) have shown a benefit of PPCI with MT over percutaneous coronary intervention alone, mainly when considering intermediate endpoints reflecting myocardial reperfusion. However, a recent series of large multicentre RCTs failed to corroborate the initial enthusiasm for MT, showing no improved benefit on hard endpoints such as mortality when compared with PPCI without MT. Furthermore, the largest RCT to date raised safety concerns after reporting an increased stroke risk after MT. We review the background, value, and implications of the current evidence before concluding that the routine use of MT in PPCI for STEMI should not be encouraged.

Keywords: ST-elevation myocardial infarction (STEMI), manual thrombectomy (MT).

## INTRODUCTION

Over the last few decades, primary percutaneous coronary intervention (PPCI) has become the preferred treatment to reduce mortality of acute myocardial infarction with ST-segment elevation (STEMI).[1] Despite successful recanalisation of the infarct related artery (IRA) with balloon angioplasty and coronary stents, restoration of coronary flow and myocardial perfusion is often incomplete. This 'no-reflow' phenomenon is associated with larger infarct size and increased mortality.[2] One of the causes of no-reflow is the distal embolisation of a thrombus, which is ubiquitous to STEMI culprit lesions. Manual thrombectomy (MT) with dedicated aspiration catheters was conceived as a strategy to reduce thrombus burden prior to coronary stenting and became part of the standard treatment strategy in PPCI, being used in 30–60% of real-world practice.[3,4] Although the principles of MT are intuitively beneficial, clinical research designed to determine the clinical value of routine MT in PPCI has provided conflicting results. We will review the available evidence for the use of the MT in PPCI.

## EARLY RANDOMISED CONTROLLED TRIALS

Initial enthusiasm for MT came from a couple of small randomised controlled trials (RCTs) that demonstrated a reduction of immediate no-reflow. The REMEDIA trial randomised 100 consecutive patients presenting with STEMI to either standard percutaneous coronary intervention (PCI) or PCI with manual thrombus aspiration.[5] This study showed that the use of MT was associated with significantly better myocardial blush grade (MBG) and ST-segment resolution (STR). These angiographic and electrocardiographic derived parameters were surrogate indicators of better microvascular reperfusion and less no-reflow. De Luca et al.[6] reported the same observations in 76 consecutive patients with anterior STEMI. These authors also reported a lower incidence of adverse left ventricular (LV) remodelling on transthoracic

echocardiography at 6 months in the MT group. The multicentre PIHRATE, EXPORT, and VAMPIRE trials reported similar improvement in no-reflow in 196, 249, and 355 patients with STEMI, randomised to MT compared with standard PCI alone, respectively.[7-9] However, none of these trials were adequately powered to answer whether MT improved clinical outcomes.

### The TAPAS Study

One example of a larger, high profile RCT reporting the clinical benefit of MT is the TAPAS trial.[10] This single-centre study randomised 1,071 patients to PPCI with MT or to conventional PPCI alone without MT. This study demonstrated better immediate MBG and STR in the MT group that appeared to translate to a clinical benefit of optimised reperfusion: the MT group had significantly less mortality and re-infarction at 30 days and less mortality at 1 year after the index STEMI;[11] however, the TAPAS trial was not powered to address these secondary endpoints. A subsequent meta-analysis of 18 clinical trials randomising patients with STEMI to an adjuvant MT device prior to PCI compared with PCI alone, including TAPAS, concluded that MT was beneficial in reducing mortality compared with PCI alone.[12] Based on these encouraging results, the European Society of Cardiology (ESC) and the American College of Cardiology/American Heart Association (ACC/AHA) guidelines initially recommended MT as adjunctive therapy during PPCI (Class IIa – level of evidence: A in ESC guidelines, B in ACC/AHA).[13,14]

## RECENT MULTICENTRE RANDOMISED CONTROLLED TRIALS

Whereas the early trial evidence favoured the use of routine MT in PPCI for STEMI, this consensus now needs to be revised after the results of a series of large multicentre RCTs, which demonstrated a neutral effect of MT on hard clinical endpoints. The INFUSE-AMI trial enrolled 452 patients with a large anterior STEMI and compared MT versus no MT and intracoronary (IC) abciximab versus no IC abciximab in a 2x2 factorial design.[15] This trial reported that MT was not effective in reducing major adverse cardiac events (MACE) at 30 days, whereas IC abciximab did reduce MACE. The TASTE trial included 7,244 patients with STEMI undergoing PPCI. These patients were randomly assigned to MT followed by PCI or to PCI only.[16] This multicentre, prospective, open-label, clinical RCT, enrolled patients from the national comprehensive Swedish Coronary Angiography and Angioplasty Registry (SCAAR). The authors reported no reduction of 30-day mortality with routine thrombus aspiration before PCI as compared with PCI alone. Finally, the multicentre TOTAL trial randomly assigned 10,732 patients with STEMI to undergo PPCI with routine upfront MT versus PPCI alone.[17] This very large study concluded that routine MT, as compared with PCI alone, did not reduce the 180-day risk of cardiovascular death, recurrent myocardial infarction, cardiogenic shock, or New York Heart Association (NYHA) Class IV heart failure.[18] Two recent meta-analyses gathered the data together, including the TASTE and TOTAL data, comprised of over 20,000 patients, and confirmed that routine MT before PPCI was not associated with significant benefit on mortality or re-infarction.[19,20] Meta-regression analysis did not identify any benefit of MT even when combined with glycoprotein IIb/IIIa inhibitors.

### Safety Concerns due to Stroke Risk

The TOTAL trial has yielded concerning results regarding the safety of MT. In light of earlier meta-analyses that identified a potentially higher stroke incidence after MT, TOTAL was the first trial pre-specifying stroke as a safety endpoint.[21] In TOTAL, routine MT was associated with an increased rate of stroke within 30 days of the procedure. The absolute numbers remain small (33/5,033 patients in MT versus 16/5,030 in the PCI alone, hazard ratio [HR] 2.06, p=0.02) but require consideration given the lack of benefit of routine MT. The 1-year follow-up results of the TOTAL trial confirmed a higher stroke risk (1.2% versus 0.7%, HR 1.66, p=0.015).[18] The mechanistic cause of increased cerebrovascular event risk in MT remains unclear, but could be attributed to embolisation of the thrombus and air after the retrieval of the aspiration catheter. In agreement with this hypothesis, a sub-analysis of the TOTAL trial confirmed that the majority of excess strokes occurred within the first 48 hours of the procedure.[22] The stroke risk between the two groups remained similar beyond the first 48 hours. There was primarily an increase in ischaemic strokes but also in haemorrhagic strokes and strokes of varying severity. After multivariate regression using the traditional risk factors for stroke, MT was determined to be an independent predictor of stroke.

### Does Thrombectomy Remove Thrombus?

The evidence provided by TOTAL is counterintuitive; we speculate that technical issues may prevent

the translation of the mechanical removal of a thrombus from the IRA into an improvement in prognosis after STEMI. It is known that the currently used MT aspiration catheters are often unable to remove a substantial load of thrombus. The TROFI trial compared optical frequency domain imaging (OFDI) in 141 patients randomised to PPCI with or without MT and showed that approximately 80% of the total clot burden quantified by OFDI remains in the IRA following MT.[23] In a sub-study of the TOTAL trial, the thrombus burden at the culprit lesion was compared in 243 patients treated with thrombectomy versus PCI alone using optical coherence tomography. This study concluded that MT did not reduce culprit lesion pre-stent thrombus burden compared with PCI alone and that both strategies were associated with low thrombus burden at the lesion site post-PPCI. This suggests that the effect of an aspiration catheter may not be much greater than dottering with a predilatation balloon. Development of more effective aspiration catheters evacuating all the thrombus safely may still be beneficial and worthy of further research.

## Does Thrombectomy Harm Microvascular Function?

Another consideration is whether the device used in the IRA causes microvascular injury rather than preventing it. To assess the impact of device therapy on microvascular function during PPCI procedure, our group set up the IMPACT trial.[24] We performed serial measurements of the index of myocardial resistance (IMR) in 41 PPCI patients randomised to MT or balloon angioplasty (BA) followed by stenting. IMR is a wire-based, invasive measure of microcirculatory function that can predict final infarct size and LV function in patients with STEMI.[25] Our data showed that in patients with partial restoration of flow in the IRA after passage of a guide wire, both MT and BA result in similar final IMR values. In a predefined sub-group with low IMR at baseline (IMR <32), both MT and BA prior to stenting resulted in a highly significant increase in IMR. This suggests that in patients with preserved microvascular function, instrumentation of the culprit vessel contributes to, rather than prevents, further acute microcirculatory injury. Hypothetically, the relatively large bore thrombus aspiration catheters could even cause more injury than a predilatation balloon.

## Do We Blame the Technique or the Device?

Another question is why the results from early, single-centre RCTs (TAPAS) are contradicted by the more recent, larger, multicentre RCTs (TASTE and TOTAL). TAPAS was a trial conducted in a single, high volume PPCI centre with short door-to-balloon times. Could it be considered that more diverse operator skill, PCI techniques, and patient populations of multicentre trials account for the neutral effect of MT observed in TASTE and TOTAL? A TASTE trial sub-study showing no difference in mortality, recurrent myocardial infarction, or stent thrombosis between the type of aspiration catheter used, the stent type used, the practice of direct stenting, or the use of post-dilatation balloons seems to suggest this is not the case.[26]

## Is There Any Group That Might Benefit from Manual Thrombectomy?

Sub-group analyses of TASTE and TOTAL did not identify any specific sub-group (e.g. anterior myocardial infarction or high thrombus burden) that benefitted from routine MT. MT may improve visualisation, particularly in those patients with a poor Thrombolysis In Myocardial Infarction flow where it has been shown to result in fewer and more appropriately sized stents implanted by direct stenting, which do not require further post-dilatation. However, these procedural differences did not impact clinical outcomes, at a relatively short clinical follow-up of 2 years.[27] Many of the trials suffer from selection bias; they did not randomise patients with extremely heavy clot burden, a group where MT may still have a role. In addition, improved visibility following MT may make the intervention easier and provisional use of MT if there is persistent clotting despite PCI may still be warranted. Therefore, selective or bail-out MT still receives a Class IIb recommendation, level of evidence: C in the updated ACC/AHA/SCAI guidelines.[28]

## Do We Need to Wait Longer to See the Benefits?

The aforementioned RCTs do not report follow-up beyond a year. One may suggest that the observed improvements in MBG and STR in the MT sub-groups may take more time to turn into a clinical advantage. However, this is not confirmed by the results of two recently published large observational cohort studies. Jones et al.[4] reported on the outcomes of 10,929 STEMI patients treated with PPCI. One-third (32.7%) underwent MT during PPCI. MT was used more frequently in younger patients and patients with a worse post-infarct LV ejection fraction. MT was associated with a higher procedural success rate and a lower risk of

in-hospital MACE. The median follow-up duration was 3 years (interquartile range: 1.2–4.6). However, after multivariate analysis and propensity matching, the use of MT was not associated with an improvement in long-term mortality.[4] Similar results were reported by Watanabe et al.[29] in a cohort study of 5,429 STEMI patients in Japan. These registries suggest that the results of the RCTs can be extrapolated to a 'real-world' population.

## CONCLUSION

In the past, MT with a dedicated aspiration catheter was often attempted to improve visualisation and the ease of PPCI for STEM-I. Although early evidence from RCTs revealed improvement in surrogate markers of microvascular reperfusion, large, multicentre RCTs have not been able to confirm significant clinical benefit from routine MT in PPCI. The potential for harm with a higher incidence of stroke after MT compared to PCI alone now clearly discourages the routine use of MT in PPCI. This is reflected in the focussed update on primary PCI for patients with STEMI guidelines recently published by ACC/AHA/SCAI, where routine MT received a Class III, no benefit, level of evidence: A, indication.[28] However, it may still have a role in selected situations e.g. particularly large thrombus burden, to improve visualisation, and as a bail-out. Enthusiasm for MT may be reinvigorated when more sophisticated and effective catheters are conceived in the future. The rise and fall of the use of MT confirms, once more, that the findings of small studies using intermediate endpoints must be substantiated by large RCTs assessing meaningful clinical endpoints before wider adoption is endorsed.

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# TREATMENT OPPORTUNITIES FOR COLORECTAL LIVER METASTASES

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**Disclosure:** The author has declared no conflicts of interest.
**Received:** 05.02.16 **Accepted:** 10.03.16
**Citation:** EMJ. 2016;1[2]:76-83.

## ABSTRACT

Colorectal liver metastases (CLM) are the most common hepatic malignancy and are caused by disseminated tumour cells (DTCs) seeded early in the tumourigenesis of colorectal cancer. Despite optimal treatment, CLM are associated with high mortality rates. This review provides an overview of three promising strategies to extend survival in CLM: treatment of DTCs, immunotherapy, and new surgical resection techniques.

Keywords: Colorectal liver metastases (CLM), circulating tumour cells, immunotherapy, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), resection and partial liver segment II/III transplantation with delayed total hepatectomy (RAPID).

## INTRODUCTION

Colorectal cancer (CRC) is the second most common cancer in Europe, with an estimated 447,000 new cases and 215,000 deaths in 2012.[1] Distant metastasis (advanced CRC) is the major cause of CRC-related death, and ultimately develops in 50% of patients with CRC.[2] The liver is the most common metastatic site and is involved in approximately 70% of cases.[3] Colorectal liver metastases (CLM) are thereby the most common hepatic malignancy.[4] When CLM are left untreated, median survival rarely exceeds 9 months.[5] Improvements in treatment over recent decades have increased survival, but so far disease-specific 10-year survival in advanced CRC remains ~5%.[6]

CRC spreads to the liver via the blood as disseminated tumour cells (DTCs), and the metastases that arise are difficult to treat, especially in the liver. This review provides an overview of three strategies that can potentially prolong survival in patients with CLM: treating the DTCs, immunotherapy, and new techniques allowing larger liver resections.

## DISSEMINATED TUMOUR CELLS

Shedding of cells to the circulation does not exclusively occur in malignancy; circulating epithelial cells are found in various benign conditions.[7] However, in contrast to their benign counterparts, some DTCs can extravasate and colonise distant sites. The low concentration of DTCs (approximately 2 cells per mL are usually expected) makes them difficult to detect in peripheral blood, and even with current technologies the median DTC detection rate in advanced CRC is ~35%.[8] Preclinical studies suggest that the concentration of DTCs is high, even in early phases of cancer development.[9] More than a decade ago, Flatmark and colleagues[10] sampled bone marrow at the time of CRC resection and detected DTCs in 10% of the patients with Stage I CRC. Similarly, by sampling mesenteric venous blood during CRC resection, Seng et al.[11] found DTCs in almost all patients with Stage I CRC. However, DTCs were also detected in the bone marrow of a small number of patients with colorectal adenomas.[10] In a breast cancer study of similar design, DTCs were also detected in the bone marrow of patients with premalignant lesions.[12] Consequently, the mere presence of DTCs in bone marrow may have limited prognostic value; in breast

cancer, a pooled analysis revealed that two-thirds of these patients have a good prognosis.[13] Taken together, this suggests that DTCs from CRC are probably a heterogeneous population in which only a subset correspond to an adverse prognosis.

By comparing the genetic footprint of primary CRCs and their liver metastases, many studies have found a remarkable degree of similarity.[14-16] It is therefore probable that the DTCs responsible for metastasis share the mutational pattern of their parental tumour. However, despite extensive research, no metastasis-specific mutation(s) has been found; the same genetic alterations that cause malignant transformation also confer metastasis potential.[17] Two mutations in CRC, however, are particularly associated with a distinct metastasis pattern: mutations in the *KRAS* oncogene (occurring in 30–50% of patients with advanced CRC) have a higher probability of metastasising to the lungs, and are associated with increased recurrence rates following resection; while mutations in the *BRAF* oncogene (occurring in 5–15% of patients with advanced CRC) are associated with increased peritoneal and distant lymph node metastasis.[18]

CRCs can be categorised based on the type of genomic instability they display. Within this, approximately 60–70% display chromosomal instability (CIN), whereas 10–15% display microsatellite instability (MSI).[19,20] CIN cancers have a higher recurrence rate compared with diploid cancers (a recent study reported rates of 43% versus 22% in Stage II CRC),[19] while MSI cancers harbour a significantly higher number of mutations, are more immunogenic, and have lower recurrence rates.[21]

Taken together, certain mutation patterns and types of genomic instability may increase risk of metastasis, but they cannot predict or explain metastasis in a meaningful way. Other mechanisms must be responsible.

It is known that a large percentage of DTCs die before landing at distant organs, but a subset of DTCs have been found to have stem-cell-like properties. These properties include an epithelial to mesenchymal phenotype, increased capacity for migration and invasion, and resistance to apoptosis.[22] CD133 may be a promising marker for these properties in CRC as higher percentages of CD133$^+$ cells are detected in liver metastatic tissues than in primary tumours.[23] High levels of CD133 in the primary tumour is associated with shorter overall survival,[24] and a high number of CD133$^+$ DTCs in mesenteric blood at the time of CRC resection is associated with relapse.[11]

The ability of different organs to support CRC DTC growth is variable, as is evident from the relatively high incidence of DTCs in the bone marrow, but incidence of actual bone metastasis in advanced CRC is low. This suggests that CRC DTCs survive in the bone marrow in a non-proliferative state.[25] The liver, on the other hand, may be a more 'permissive' environment for DTC growth.[25] A recent, preclinical study has demonstrated that DTC growth in the liver is facilitated by certain tumour-derived vesicles called exosomes.[26] Exosomes encoding the integrin αvβ5 were found to specifically bind Kupffer cells in the liver, upregulating pro-inflammatory signalling pathways and increasing liver metastasis. Consequently, by deploying integrin-blocking decoy peptides, the liver metastasis was successfully ablated. Finally, the authors showed that αv expression in patient-derived exosomes could predict metastasis to the liver.[26]

The process of colonisation by CRC DTCs also elicits inflammation in the liver.[27] An example is the activation of the bone morphogenetic protein (BMP) signalling pathway,[28] which is usually considered tumour suppressive.[29] However, certain DTC mutations (including, but not limited to, *SMAD4*) change the effects of this pathway, allowing or even stimulating growth of DTCs in the liver when BMP activity is increased.[29,30] A similar pattern is observed for transforming growth factor-β signalling;[31] certain mutations thus enable DTCs to thrive in otherwise unfavourable conditions.

An increasing body of evidence suggests that the DTCs, when arriving in the liver, lodge in an area around the capillaries named the perivascular niche.[32] This niche is highly similar to, and possibly the same as the niche wherein tissue-resident stem cells reside.[32] In patient-derived CLM specimens, CD133$^+$ cells were found to be concentrated around the microvasculature.[33] It is proposed that the endothelial cells mediate DTC survival here as CD133$^+$ CRC cells were found to be highly resistant to the chemotherapeutics fluorouracil and oxaliplatin *ex vivo* when the culture medium was conditioned by liver endothelial cells. However, without the conditioning, the cells died.[33]

There are several conceivable strategies for dealing with the DTCs in the perivascular niche, for

example, given their reliance on angiogenesis for growth, it is conceivable that a continuous administration of anti-angiogenic drugs could maintain the dormancy of the DTCs here.[34,35] The efficacy of an mTOR inhibitor for this purpose has been demonstrated pre-clinically.[36] Alternatively, blocking endothelial-derived resistance factors, such as the Notch ligand,[33] possibly in combination with chemotherapeutics, are another option. For instance, a recent Phase I study of a Notch inhibitor demonstrated tolerability of the drug in advanced cancer.[37] Lastly, interfering with pathways that are affected by DTC mutations may also be necessary. For *SMAD4* mutated cancers, the Rho-kinase inhibitor fasudil may be a candidate drug.[30]

## IMMUNOTHERAPY

Tumour cells are constantly identified and eradicated by the immune system. Patients with a suppressed immune system, for example those taking immunosuppressive drugs following an organ transplant, or HIV-positive patients, have an increased incidence of malignancies.[38] Consequently, it is conceivable that stimulating the immune system in a specific fashion can lead to tumour rejection and elimination. More than 30 years ago, Rosenberg and colleagues[39] showed that administration of interleukin-2 (a T cell growth factor) can cause durable regression of metastatic disease, one of the first demonstrations of this principle.

T cells have been a major focus in immunotherapy due to their capacity for selective recognition of peptides (antigens), their capacity to directly kill antigen-expressing cells, and their ability to orchestrate diverse immune responses involving both the adaptive and innate immune system.[40] The process of T cell activation first involves the T cell receptor binding to an antigen presented on a major histocompatibility complex molecule on the surface of an antigen-presenting cell. Following this, co-receptors and co-ligands, as well as adhesion molecules, assemble to form what is known as an immunological synapse.[41] In this synapse, co-stimulatory and co-inhibitory signals (immune checkpoints) ultimately define the T cell response.[41] Some of the important receptors and ligands in this synapse are schematically illustrated in Figure 1.

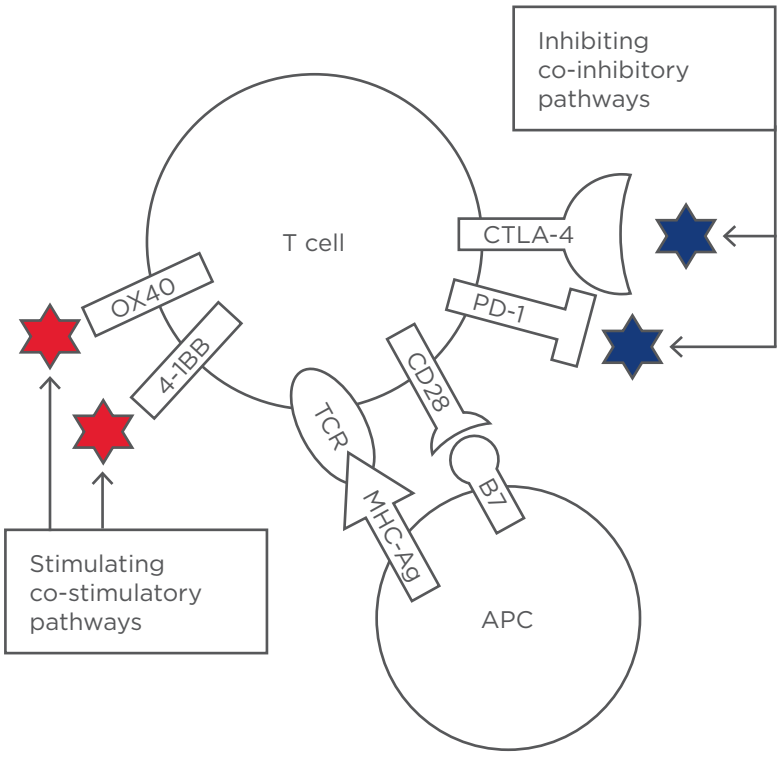


**Figure 1: Druggable co-signalling receptors in the immunological synapse. The goal is to evoke an endogenous anti-tumour response.**

MHC-Ag: major histocompatibility complex - antigen; APC: antigen presenting cell; TCR: T cell receptor; CTLA-4: cytotoxic T lymphocyte-associated protein-4; PD-1: programmed cell death protein-1.

Essentially, genetic and epigenetic alterations in tumours provide antigens that distinguish the tumours from normal cells. In order to evade immune destruction, tumours evolve several mechanisms to reduce their immunogenicity. The overexpression of inhibitory ligands and receptors that regulate T cell effector functions is one such mechanism.[41]

Monoclonal antibodies (mABs) targetting cytotoxic T-lymphocyte associated protein (CTLA)-4 became the first approved drug targeting a T cell checkpoint. CTLA-4 is expressed exclusively on T cells, where it surfaces upon T cell activation and competes for B7, the ligand of CD28, thereby inhibiting co-stimulation and dampening T cell effector function. Blocking CTLA-4 has shown promising results in treating advanced malignant melanoma, but has no meaningful response as a single agent in advanced CRC.[42] In contrast to CTLA-4, programmed death (PD)-1 is not solely expressed on T cells, but also on B cells and natural killer cells. PD-1's main function is to limit the activity of T cells in peripheral tissues. One of its ligands, programmed cell death-ligand 1 (PD-L1), is expressed on many tumour cells. mABs that neutralise PD-L1 have been developed, but their efficacy is primarily restricted to cancers that express, or have immune-infiltrating cells expressing PD-L1.[43] Recently, an objective response was achieved in 4 out of 10 patients with in MSI-advanced CRC receiving anti-PD-L1.[44]

In addition to blocking co-inhibitory receptors, enhancing anti-tumour immunity by targeting co-stimulatory molecules is also a possibility. Upon ligation, the tumour necrosis factor (TNF) superfamily member 4-1BB receptor evokes robust effector T cell responses.[45] A 4-1BB agonist is currently being evaluated in combination with an epidermal growth factor receptor (EGFR) antagonist in advanced CRC.[46] Another promising receptor in the TNF superfamily is OX40. High OX40 expression has been linked to a favourable outcome in CRC.[47] Clinically, in a mixed population of metastatic solid tumours, response was achieved in at least 1 tumour nodule in 12 out of 30 patients given an OX40 agonist.[48] A clinical trial using mABs stimulating OX40, in combination with liver resection in advanced CRC, is currently recruiting patients.[49]

The mutational load of a tumour seems to predict response to immunotherapy.[50] This translates to MSI tumours for patients with advanced CRC. Furthermore, it is likely that the key to success lies in combinations of checkpoint drugs: a combination of anti-PD-L1 and anti-CTLA-4 is currently being evaluated in MSI CRC patients,[51] however, immunotherapy is only effective if an immune response is present prior to therapy.[40] It may be necessary to combine these strategies with therapies that induce *de novo* immune responses, such as vaccines.[40] Alternatively, a readily available therapy is the combination of conventional radiotherapy or chemotherapy with immunotherapy. Preliminary results from a study combining anti-PDL1 with conventional chemotherapy and anti-VEGF in patients with advanced CRC achieved objective responses in 12 out of 30 patients.[52] Finally, recent studies have also suggested that cells of the innate immune system can be modulated to play a role in combatting cancer.[53] It is likely that, in order to make immunotherapy widely applicable, not only must T cells be stimulated, but antigen-presenting cells also.[53]

## SURGERY

The liver is the sole organ with metastases in approximately a third of patients with advanced CRC, but traditionally only ~20% present with resectable disease.[54,55] The rationale for trying to increase resectability lies in the dramatic survival benefit as 5-year overall survival approaches 50% after surgery, while for chemotherapy the 5-year survival rate is approximately 10%.[56,57]

The liver has eight segments, each with a portal vein, hepatic artery, bile duct, and hepatic vein branches. This means that they can be resected individually, leaving the other segments uncompromised.[58] The liver is a solid parenchymal organ, which makes its division somewhat challenging. The traditional finger fracture technique for transecting the parenchyma has been replaced by newer surgical instruments, such as the ultrasound aspirator, water jet dissector, or LigaSure™. Combined with surgical staplers for the larger vessels, good cooperation with the anaesthetist to ensure a low central venous pressure and, if needed, clamping of the blood flow into the liver (Pringle manoeuvre) means that liver surgery can currently be performed with very little blood loss.

Traditionally, CRC is resected, followed by chemotherapy and hepatic resection of CLM. In the time period between the colorectal and hepatic resection(s), the CLM may continue to grow, in some

cases rendering the CLM unresectable. This is a pressing concern when there are complications following CRC surgery that delay chemotherapy and any subsequent hepatic resection(s). A decade ago, a 'liver-first' strategy was proposed as a possible solution.[59] In this approach, systemic chemotherapy is followed by resection of synchronous CLM, and following this, resection of the primary colorectal tumour. Advantages include possible downstaging of both primary tumour and liver metastases prior to surgery and avoidance of time loss. The liver-first approach is feasible, but an effect on patient survival has not yet been demonstrated.[60,61]

The liver has a unique ability to regenerate, but approximately 20–25% of the total functional liver volume should remain as a minimal remnant (and potentially more if there is chemotherapy-induced liver injury)[62] to avoid post-hepatectomy liver failure. Recently, a new technique for hepatic resection has been described, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS; Figure 2).[63]

The ALPPS technique relies on the fact that ligation of the portal vein to a lobe of the liver results in atrophy of the corresponding lobe, and hypertrophy of the other. The hypertrophy may partly be due to increased portal blood flow, but the procedure also increases levels of proinflammatory mediators and potentially levels of growth factors, which may also be a contributing factor.[64] ALPPS is most commonly performed to allow a safe resection of a large part of the right liver. In the first part of the procedure, the right liver is mobilised by dividing accessory hepatic veins. Next, the common bile duct, left and right hepatic artery, right branch of the portal vein, portal vein branch for segment IV, and the right hepatic vein are identified. The right portal branch and the branch for segment IV are subsequently isolated and ligated. Following this, the liver parenchyma is transected all the way to the anterior surface of the inferior vena cava (Figure 2A).[65,66] Finally, the arterial supply and the venous drainage of the right liver are kept intact, the right liver is left *in situ*, and the wound closed.

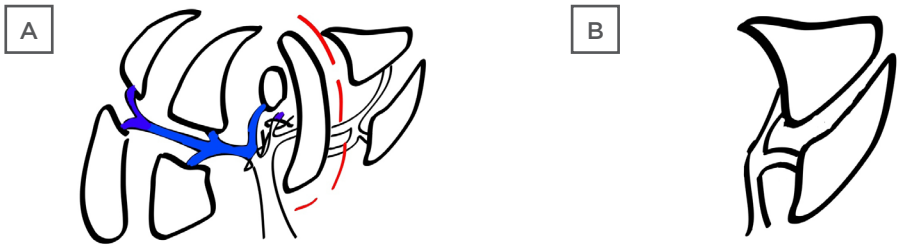


**Figure 2: The ALPPS procedure.**
A: The right portal vein and the portal branch to segment IV is ligated. The blue areas indicate stasis of blood. The liver parenchyma is transected along the dotted red line. B: Status post-hepatectomy (only the portal vein is depicted).
ALPPS: associating liver partition and portal vein ligation for staged hepatectomy.

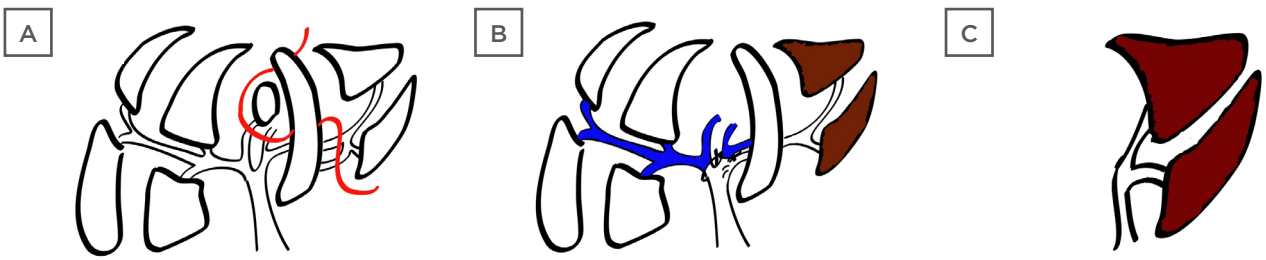


**Figure 3: The RAPID procedure.**
A: Liver segments I–III are resected along the red line. B: A segment II/III donor graft, coloured brown, is transplanted. Subsequently, the portal vein branches to the remaining liver are divided, the blue areas indicate stasis of blood. C: Status post-hepatectomy (only the portal vein is depicted).
RAPID: resection and partial liver segment II/III transplantation with delayed total hepatectomy.

A computed tomography scan is performed 5–7 days later; if the remnant liver (the left liver) has increased to ~30% of the total liver volume, the second step of the procedure is performed. In the second step, the right hepatic vein, right hepatic artery, right branch of the bile duct, and arterial branch to segment IV are identified, ligated, and divided. The right liver is then removed (Figure 2B).[65,66] Commonly an increase of ~70% is achieved in the remnant liver after 1 week.[66] The procedure can be performed with very low morbidity and mortality.[66] ALPPS enables large liver resections; and large resections, e.g. leaving just one segment of the liver, have been reported in the literature.[67]

Motivated by the large differences in survival between surgical resection and chemotherapy for CLM, Hagness et al.[68] evaluated liver transplant in 21 patients with unresectable CLM. The 5-year estimated overall survival was 56% in these patients (median follow-up, 65 months).[68,56] In a similar cohort of patients receiving first-line chemotherapy, the corresponding 5-year overall estimated survival was 9% (median follow-up, 60 months).[56]

A shortage of organ donors will limit the application of whole-organ liver transplantation for CLM; however, a strategy with the potential to circumvent the shortage of organ donors has recently been proposed (Figure 3): the Resection And Partial liver segment II/III transplantation with Delayed total hepatectomy (RAPID) procedure.[69] In this case, liver segments I–III are resected in the first part of the procedure, avoiding tumour-affected parts (Figure 3A). Thereafter, segment II/III of a donor graft is transplanted, anastomosing the graft liver vein to the vena cava, the portal vein, and the hepatic artery of the graft in an end-to-side fashion to the main portal trunk and the common hepatic artery, respectively. Then, similarly to ALPPS, the portal branches to the remaining liver are ligated (Figure 3B). Care is taken to avoid portal hypertension (>20 mmHg) in the graft; if needed, the right portal branch may be banded (instead of ligated) to achieve subtotal stenosis as opposed to complete occlusion. After a period of approximately 2 weeks, the second-stage hepatectomy may be performed (Figure 3C).[69]

With the RAPID approach, the prospect of living donation segment II/III grafts to patients with liver-only advanced CRC is conceivable.

## CONCLUDING REMARKS

Although the mean age upon diagnosis of CRC is approximately 70 years[10] and CRC is generally considered a disease of the old, a significant proportion of the afflicted are considerably younger. Half of CRC patients develop metastases, and in this population 50% die within 2 years,[56] a testament to the considerable potential for improvement. Many hoped that whole cancer genome sequencing would identify key mutations responsible for tumour aggressiveness, but the key feature that emerged may be the immense heterogeneity of cancer.[17] Currently, the only application of cancer genomics in CRC is ruling out those with mutations in *RAS* and *BRAF* for anti-EGFR therapy, but perhaps accumulating data from transcriptomics and proteomics will change this over time. Pragmatically, mutational analysis of a limited number of genes seems sensible, perhaps even in the entire CRC population. Additionally, identifying patterns of genomic instability, as well as the expression levels of some key markers, such as PD-L1, CD133, and as recently suggested, CDX2,[70] may help identify patients with risk of relapse or those that might benefit from adjuvant chemo or immunotherapy.

Indeed, immunotherapy offers great hope, but presently it remains to be seen whether this will improve long-term survival, and whether its use will be limited to particular patient groups.[71] Stimulating T cells inherently carries risk of auto-immunity; the potential for fatal pneumonitis or even hepatitis is a concern.[71]

Removing the tumour load in the liver in advanced-CRC patients has a dramatic effect on survival, as recently demonstrated by the pilot trial of liver transplantation for unresectable CLM.[56] Interestingly, of the 21 patients transplanted, not a single patient had the liver as the first site of recurrence.[56] This is in contrast to the situation after liver resection, where approximately 40% of patients have the liver as the first site of recurrence.[56] These observations may, at least in part, be attributed to microscopic, undetectable tumour residue in the remaining liver, perhaps in the form of dormant DTCs. One may further speculate whether the mTOR-based immunosuppression in transplanted patients is suppressing the growth of DTCs. Taken together, one may argue that maximum surgical debulking in advanced CRC, even without a tumour-free result, may be the way forward.

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# ALCOHOLIC LIVER DISEASE: A COMPREHENSIVE REVIEW

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 17.11.15 **Accepted:** 02.02.16
**Citation:** EMJ. 2016;1[2]:85-92.

## ABSTRACT

Alcoholic liver disease, a leading cause of morbidity, mortality, and cirrhosis, can range from simple steatosis to hepatocellular carcinoma. Multiple mechanisms such as oxidative stress, mitochondrial dysfunction, and alteration in gut-liver axis have been proposed for the pathogenesis of alcoholic liver disease. Based on different prognostic models, alcoholic hepatitis patients can be stratified into sub-groups and specific pharmacological therapy can be started. Alcohol abstinence has a clear cut mortality benefit and nutritional support is very important as most of the patients are malnourished and in a hypercatabolic state. Other than conventional glucocorticoids and pentoxifylline, newer agents and combination therapy can be used in severe alcoholic hepatitis in patients not responsive to conventional glucocorticoid therapy. Liver transplantation improves survival in advanced alcoholic cirrhosis and it can be an option in severe alcoholic hepatitis patients who are not responding to other medical therapies. Whether early transplantation can improve the survival compared with the conventional waiting period of 6 months is an active area of investigation. This is due to the fact that most of the disease-related mortality occurs in the first 2 months.

Keywords: Alcoholic hepatitis, alcoholic liver disease, cirrhosis, steatohepatitis, hepatocellular carcinoma (HCC), liver transplantation.

## INTRODUCTION

Alcohol related toxicity is the third most common cause of morbidity[1] and the fifth most common cause of disease burden worldwide.[2] Alcohol abuse is the leading cause of mortality in people aged 15–49 years, and the total expenditure amounts to billions of dollars.[2] In developed countries, alcohol is the most common aetiology of cirrhosis.[3] The National Institute on Alcohol Abuse and Alcoholism recommends that both males and females should not drink more than 28 g and 14 g per day, respectively.[4]

## NATURAL HISTORY OF ALCOHOLIC LIVER DISEASE

Histological abnormalities occurring in alcoholic liver disease can range from steatosis to hepatocellular carcinoma (HCC) (Figure 1):[5]

- Hepatic steatosis: 90–95% of heavy alcohol drinkers develop macrovesicular steatosis in the centrilobular area (Zone 3).[5] Patients are usually asymptomatic. Although reversible, cirrhosis may develop in 10% of heavy drinkers[6]
- Steatohepatitis: 10–35% of heavy drinkers develop necroinflammation along with steatosis, known as steatohepatitis or alcoholic hepatitis.[6] An estimated 40% of patients with alcoholic hepatitis develop alcoholic cirrhosis; this entity has high short-term mortality and can cause portal hypertension in the absence of cirrhosis[6-8]
- Cirrhosis: 8–20% of chronic alcoholics develop micronodular or Laennec's cirrhosis.[8] Secondary factors that accelerate the progression to cirrhosis are: patterns of alcohol drinking (chronic daily heavy drinkers more than binge drinkers),[9] female gender (due to low levels of gastric alcohol dehydrogenase, and higher body fat proportion and oestrogen levels),

obesity,[10] genetic polymorphisms,[11] and comorbid conditions such as infection with hepatitis B or C virus and/or human immunodeficiency virus and haemochromatosis[5,12]

- HCC: 1.5% of patients with cirrhosis of any aetiology develop HCC[13] and 3–10% of alcoholic cirrhosis patients ultimately develop HCC

## PATHOGENESIS OF ALCOHOLIC LIVER DISEASE

### Ethanol Metabolism

Alcohol is metabolised to acetaldehyde by both alcohol dehydrogenase (at low alcohol concentrations) and CYP2E1 (at higher concentrations, >10 mM), which is further metabolised by aldehyde dehydrogenase to acetate. Acetaldehyde forms protein adducts which causes hepatocyte injury directly or by autoimmune reaction.[14]

### Oxidative Stress

Excess pro-oxidants (i.e. NAD phosphate oxidase and inducible nitric oxide synthase) in Kupffer cells, and a decrease in antioxidants (selenium, glutathione, vitamin E),[14,15] causes protein, lipid, and DNA oxidation, and causes direct cell injury by DNA damage, lipid peroxidation, and tumour necrosis factor (TNF) production signalling via nuclear factor kappa B.[14,15]

### Mitochondrial Dysfunction

Hepatocyte mitochondria are devoid of catalase and are protected from oxidative stress by the transport of glutathione from cytosol, which is impaired in alcoholic liver disease.[16]

### Hypoxia

In alcoholic liver disease, liver specific hypoxia inducible factors (HIF) are upregulated which leads to steatosis, and intestinal HIF is down regulated, which leads to increased intestinal permeability and endotoxaemia (this underscores the role of probiotics containing *Lactobacillus GG*, which preserve intestinal HIF).[17]

### Impaired Proteasome Function

Impaired proteasome function leads to an accumulation of damaged protein in the cells known as Mallory–Denk bodies. Interleukin (IL)-8 and IL-18 from dead hepatocytes propagate hepatocyte injury.[18]

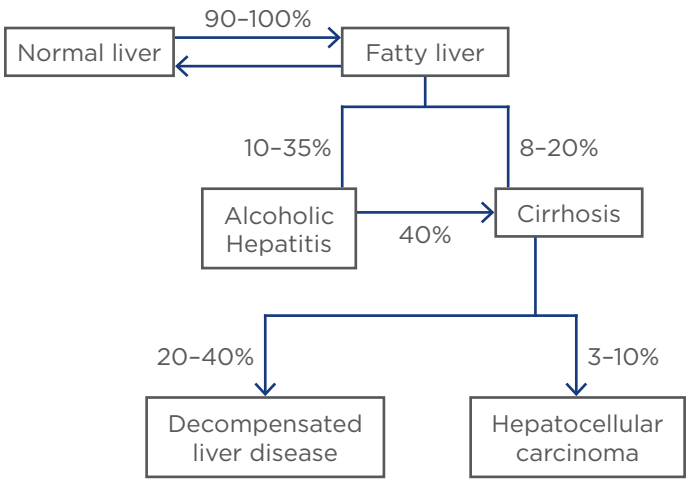


**Figure 1: The spectrum of alcoholic liver disease. Percentages represent the proportion of patients who progress from one stage to the next.**

### Abnormal Metabolism of Methionine, S-Adenosylmethionine, and Folate

S-adenosylmethionine (SAMe) is produced from methionine catalysed by methionine adenosyltransferase (MAT), and is further converted into S-adenosylhomocysteine (SAH) and homocysteine, which are toxic to the liver. Methionine can be regenerated by betaine and folic acid via 5-methyltetrahydrofolate (5-MTHF). Reduced functional activity of MAT leads to deficiency of SAMe, which maintains levels of mitochondrial glutathione (an antioxidant).[19]

### Gut-Liver Axis

Chronic alcoholism causes increased growth of gram negative bacteria and diminished levels of *Bifidobacterium spp*. and *Lactobacillus spp*.[20] in the gut, and also increases intestinal permeability by depletion of the zonula adherens protein, ZO-1, at the tight junctions. The lipopolysaccharides of gram negative bacteria cause activation of Toll-like receptors and increase production of cytokines like TNF in the liver; this results in hepatocyte injury.[14,20]

### Fibrosis

Quiescent stellate cells are transformed into myofibroblasts (by tumour growth factor β via Toll-like receptor 4 signalling), which produces collagen. Impaired fibrinolysis and accumulation of extracellular fibrin in the sinusoids cause hepatocyte hypoxia and progressive fibrosis.[21]

**Table 1: Alcohol content in different beverages.**

| Beverage | Volume (mL) | Alcohol content (g) |
|---|---|---|
| Whisky | 30 | 10 |
| Wine | 100 | 10 |
| Beer | 250 | 10 |

## DIAGNOSIS OF ALCOHOL ABUSE

Screening strategies are important as most patients are identified in the cirrhotic stage, and women of childbearing age, teenagers, and the elderly are often undiagnosed. Screening tools include the 3-item AUDIT-C (Alcohol Use Disorders Identification Test consumption questions); and the 4-item CAGE (need to Cut down, Annoyed by criticism, Guilty after drinking, need for an Eye-opener in the morning) questionnaire;[22,23] 10-item AUDIT; single question to identify risk drinking: 'How many times in the past year have you had x or more drinks a day?' (x=5 for men, 4 for women); and specific tools for pregnant women.[22,23]

There are several types of objective evidence used to diagnose alcohol abuse. These include blood and breath alcohol measurements, with the highest sensitivity and specificity being for recent drinking, but not for remote drinking due to the short half-life of ethanol. Another example is carbohydrate-deficient transferring, which has increased sensitivity when combined with mean corpuscular volume and gamma-glutamyl transpeptidase.[24] Phosphatidylethanol and ethyl glucuronide can also be used as a promising biomarker for recent alcohol abuse, and urinary ethyl glucuronide[25] is useful for monitoring in patients before and after liver transplant. Modern transdermal sensors are also used.[26]

## DIAGNOSIS OF ALCOHOLIC LIVER DISEASE

### History and Clinical Picture

The consumption of 40–80 g/day of alcohol in men and 20–40 g/day of alcohol in women for 10–12 years is required for significant risk of liver disease (Table 1).[4] Patients with fatty liver are asymptomatic at presentation, whereas most patients with alcoholic hepatitis present with jaundice and other constitutional symptoms.[27,28]

Clinically tender hepatomegaly, hepatic bruit (likely due to increased blood flow in hepatic artery), jaundice and ascites (in 60% of patients), and hepatic encephalopathy (in severe disease) can be seen in patients with alcoholic hepatitis. The liver becomes hard and smaller in size with progression of liver disease. Approximately 30% of cirrhotic patients have ascites.

In alcoholic cirrhosis, ascites is the initial pattern of decompensation compared with HCC in non-alcoholics, although ascites does not predict higher mortality, as in non-alcoholics.[27-29] The presence of stigmata of chronic liver disease in these patients (spider angioma, palmar erythema, gynecomastia, parotid and lacrimal gland enlargement, muscle wasting, and Dupuytren's contractures) usually suggests underlying alcoholic cirrhosis.[27,29]

### Investigations

In alcoholic hepatitis, aspartate aminotransferase (AST) levels are <300 U/L, alanine aminotransferase (ALT) is only mildly elevated and the AST/ALT ratio is typically >2 (ALT synthesis in the liver requires pyridoxal phosphate, more so than AST synthesis). Alcoholic hepatitis is typically associated with elevations in serum hepatic alkaline phosphatase and gamma-glutamyl transpeptidase, and with hyperbilirubinaemia.[14,27,28]

Liver biopsy in alcoholic hepatitis shows swollen hepatocytes containing amorphous eosinophilic Mallory bodies surrounded by neutrophils,[7] and intra-sinusoidal fibrosis is characteristic, which can lead to sclerosing hyaline necrosis (i.e. obliteration of the terminal hepatic venules). Alcoholic cirrhosis is typically micronodular and may gradually transform to macronodular cirrhosis (indistinguishable from other forms of cirrhosis).[7]

### Differential diagnosis

Differential diagnosis includes non-alcoholic fatty liver disease (patients who present with metabolic syndrome and a weekly alcohol intake of <21 drinks for men and <14 for women),[7,14] hereditary haemochromatosis (presence of *C282Y* and *H63D HFE* gene mutations and a hepatic iron index value >1.9 µMol/g per year, although it can occur in alcoholic cirrhosis and iron overload) and Budd–Chiari syndrome (for which Doppler ultrasound may be useful).[30] Amiodarone hepatotoxicity may also histologically resemble alcoholic liver disease.[14]

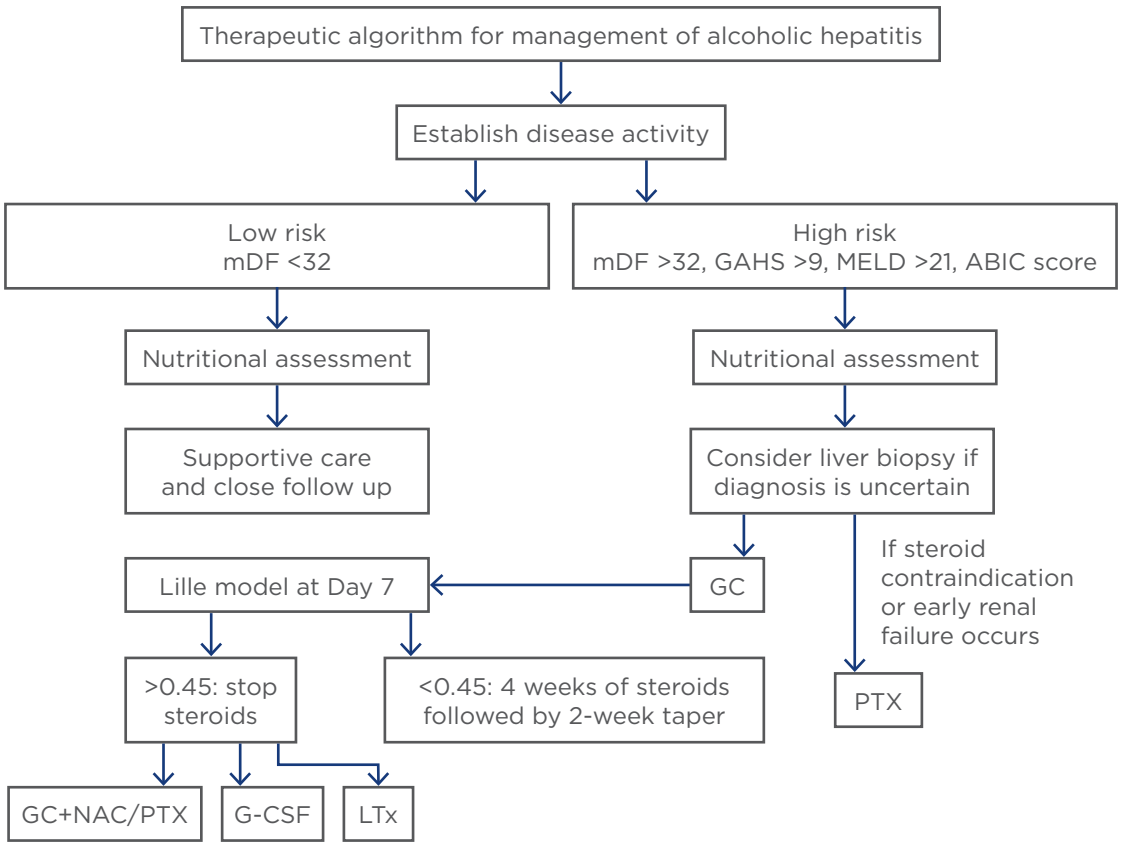


**Figure 2: Therapeutic algorithm for management of alcoholic hepatitis.**
mDF: Maddrey's discriminant function; GAHS: Glasgow Alcoholic Hepatitis Score; MELD: Model For End-Stage Liver Disease; ABIC: Age, serum Bilirubin, International normalised ratio, and serum Creatinine level; GC: glucocorticoids; NAC: N-acetyl cysteine; G-CSF: granulocyte colony stimulating factor; LTx: liver transplantation; PTX: pentoxifylline.

## ASSESSING THE SEVERITY OF ALCOHOLIC HEPATITIS

A Maddrey's modified discriminant function (mDF = 4.6 × prothrombin time [PT] − control value [seconds]) + serum bilirubin [mg/dL]) value of >32 (which indicates severe alcoholic hepatitis and warrants corticosteroid treatment) and/or hepatic encephalopathy in patients without treatment has a 28-day survival rate of 65%.[31] Non-standardisation of PT is a limitation, depending upon the type of thromboplastin used.

A GAHS (Glasgow alcoholic hepatitis score) of ≥9 at Day 1 and 7 was more accurate than mDF in predicting survival at 28 days and 84 days but is not widely validated.[32] A MELD (model for end-stage liver disease) score (based on serum levels of bilirubin, creatinine, and international normalized ratio [INR]) of ≥21 (associated with a 90-day mortality rate of 20%) is the threshold for initiating corticosteroids and is helpful in patients with alcoholic hepatitis who are candidates for liver transplantation.[33,34] Based on ABIC (Age, serum Bilirubin, INR, and serum Creatinine level) score, patients can be stratified into low, intermediate, and high-risk, with 3-month survival rates of 90%, 70%, and 25%, respectively.[35]

The stopping rule for corticosteroids is based on bilirubin levels and can be determined by the Lille score. If it is >0.45 after 7 days of corticosteroids, then it should be stopped as they have higher 6-month mortality than in patients with a score of <0.45. However, the score cannot be calculated at onset.[36] So, physicians may utilise mDF and the Lille score for initiating corticosteroid treatment and for assessing treatment response to steroids in patients with alcoholic hepatitis, respectively (Figure 2).

## ALCOHOLIC CIRRHOSIS

Five-year survival in alcoholic cirrhosis can be best predicted by Child–Pugh (CP) score

(based on serum bilirubin, albumin, PT, hepatic encephalopathy, and ascites; patients with cirrhosis can be stratified into groups A: 5–6; B: 7–8; or C: ≥9). One-year mortality in CP score A, B, and C are 15%, 25–30%, and 70–80% respectively.[33] One-year mortality is 30% in patients with alcoholic cirrhosis with ascites, 50% in those with ascites and variceal bleeding, and 65% in patients with hepatic encephalopathy.[14] Other prognostic models include the Beclere model (based on serum bilirubin, albumin, age, and hepatic encephalopathy) and MELD score (which predicts short-term survival and is used for allocation of donor livers).[14]

Acute-on-chronic liver failure can occur in stable compensated alcoholic cirrhosis, most commonly due to an increase in alcohol intake, acute viral infections (Hepatitis A, E, and B, and influenza A), and hepatotoxic drugs (paracetamol toxicity, idiosyncratic drug reactions, and herbal medicines).[37] The SOFA (sequential organ failure assessment) score can predict prognosis of hospitalised intensive care unit (ICU) patients; the 90-day mortality rate of patients who require ICU management for ≥3 failing organ systems due to infection, hepatic encephalopathy, sepsis, acute kidney injury, or multi-organ failure exceeds 90%.[38] In alcoholic liver disease, the chance of HCC is 2 to 3-times higher and co-existent hepatitis C virus (HCV) infection doubles the risk. The risk of HCC is higher in males and the elderly.[39]

## TREATMENT

### Abstinence from Alcohol

Alcohol abstinence improves liver histology and reduces the risk of a portal hypertensive bleed. Abstinence for 3 months leads to clinical improvement in 66% of patients and after 2 years, improvement is seen in laboratory parameters, such as muscle mass, and medications and diuretics can be stopped in some patients. Three years of abstinence increases survival rate to 70–80% (compared to 20–30% in those who drink heavily), but only 10% of patients maintain safe drinking after 1 year and 75% of patients relapse within a year.[40,41] Among the different medications available to treat alcohol dependence, disulfirum is the oldest, but has poor tolerability and there is little evidence that it increases abstinence. Short-term treatment with opioid antagonists such as naltrexone (available in injectable extended release form) is useful in lowering the risk of relapse. The $GABA_B$ receptor agonist, baclofen, is shown to improve abstinence and decrease relapse. Among these agents, baclofen is a relatively safe and effective agent (devoid of hepatotoxicity) in advanced cirrhosis, and is the most preferred mode of treatment.

N-methyl-D-aspartate receptor blockers are currently being investigated as a new pharmacological treatment. Alcohol abstinence support groups may also be helpful for alcohol cessation.[40-43] At the same time, the majority of patients classed as alcoholics also smoke. Smoking cessation decreases progression of hepatic fibrosis, risk of sepsis related deaths, risk of HCC, and post-transplant complications (such as hepatic artery thrombosis, cardiovascular complications, sepsis, and extrahepatic malignancies such as laryngeal, pharyngeal, and lung malignancy).[40,41,44]

### Nutritional Support

Causes of malnutrition in alcoholic liver disease include the following:

- Poor oral intake
- Nausea and vomiting
- Diarrhoea and malabsorption
- Fasting for procedures, poor palatability of foods due to low salt (in ascites), and protein-restricted diet (in hepatic encephalopathy)
- Hypercatabolism
- Effects of cytokines
- Deficiencies of vitamins (B, A, D, and E) and micronutrients (magnesium, zinc, and selenium)
- Complications of liver disease, such as ascites and hepatic encephalopathy

Depletion of hepatic glycogen stores in cirrhotic patients leads to early starvation at 12 hours (which leads to peripheral muscle proteolysis after brief starvation), compared with 48 hours in normal individuals. Therefore, protein restriction should be limited to an initial 24–48 hours in hepatic encephalopathy. Branched-chain amino acids may be substituted for standard enteral formula if the latter causes hepatic encephalopathy.[45-47]

Bedside tests for malnutrition include handgrip strength, mid-arm muscle mass, SGA (subjective global assessment) based on wasting of muscles, oedema, subcutaneous fat loss, glossitis, and cheilosis. Pre-albumin and albumin better reflect the extent of liver disease than nutrition. Altered renal function and fluid retention make body mass index and the creatinine height index unreliable markers of malnutrition.[45-47] Both malnourishment

and obesity are poor outcome predictors in liver transplantation. Psoas muscle cross-sectional area determination by computed tomography scan is an objective method to predict poor outcomes in liver transplant patients with malnourishment.[45]

The risk of severe malnutrition in CP Class A and CP Class C cirrhosis is 45% and 95%, respectively. Even in patients with stable, compensated cirrhosis, malnutrition is associated with higher 1-year mortality (20% versus 0%) and complication rates (65% versus 13%).[44]

### Nutritional Therapy in Alcoholic Hepatitis

In two large Veterans Administration studies, the 6-month mortality rate in severe alcoholic hepatitis correlated in a dose-response fashion with voluntary dietary intake. Despite this, two-thirds of the patients failed to consume the recommended caloric intake of 2,500 kcal/day. Therefore, there should be hesitation in placing a nasogastric feeding tube if the patient cannot voluntarily ingest at least 2,500 kcal/day, even when oesophageal varices are present. Glucocorticoid therapy can increase voluntary dietary intake, but providing adequate calories through enteral feeding provides the same 1-month survival benefit with significantly lower mortality at 1 year.[16,45-47]

## TREATMENT OF ALCOHOLIC HEPATITIS

### Glucocorticoids

In severe alcoholic hepatitis with mDF >32 or spontaneous hepatic encephalopathy (with no contraindications to steroids, no active gastrointestinal bleeding, serum creatinine ≤175 µmol/L, no active infectious process, or underlying chronic renal insufficiency), 28 days of methylprednisolone followed by a 2-week taper (Figure 2) reduced short-term mortality from 35% to 6%, and from 47% to 7% in patients who had hepatic encephalopathy at onset.[48,49] The interruption of treatment with corticosteroids can be based on Lille score. If the Lille score is >0.45 after 7 days of corticosteroid treatment, treatment should be stopped as 6-month survival is estimated at 25% contrary to patients with a Lille score below this cut-off (85%). The 2-month mortality of patients on glucocorticoid in most of the trials is 20–30%. In an active infection, glucocorticoid can be used if there is rapid clinical improvement (as shown in a prospective study), otherwise 25% of patients will not be considered for therapy.[50] Twenty-five percent of patients are steroid resistant, and have a 6-month survival rate of 25%.[50]

### Pentoxifylline

In patients with severe alcoholic hepatitis, in whom glucocorticoids cannot be used, pentoxifylline is an effective alternative that has been shown to have short-term mortality (30-days) benefit, and to reduce incidence of hepatorenal syndrome.[51] Renal failure as a cause of death in patients with cirrhosis is remarkably low in the patients treated with pentoxifylline (10% compared with 70%).[51] In steroid non-responders (according to the Lille model), switching to pentoxifylline (Figure 2) rather than continuing glucocorticoid treatment does not increase short-term mortality.[52] In another head-to-head trial, pentoxifylline had lower short-term (2 months) mortality (15% compared with 35%) and incidence of hepatorenal syndrome, compared with glucocorticoids in patients with severe alcoholic hepatitis.[53] In a large (n=1103) multicentre, double-blind, randomised trial investigating whether prednisolone and/or pentoxifylline are effective in alcoholic hepatitis, pentoxifylline did not improve survival. Prednisolone was associated with a reduction in short-term mortality without any effect on intermediate and long-term mortality.[54]

### Combination Therapy and Comparative Efficacy

Glucocorticoids plus N-acetyl cysteine therapy for 5 days have lower short-term (30 days) mortality compared with glucocorticoids alone, but had increased incidence of hepatorenal syndrome with no difference in medium-term mortality (90–180 days).[55] Glucocorticoids and pentoxifylline combination therapy have similar mortality but a lower incidence of hepatorenal syndrome as cause of death compared to glucocorticoids alone.[56] Comparative efficacy of pharmacological agents and network analysis has shown that in severe alcoholic hepatitis, glucocorticoid monotherapy or combination therapies and pentoxifylline reduce short-term mortality without any decrease in medium-term mortality.[57]

### Newer Agents

In a new randomised, pilot study, granulocyte colony stimulating factor has emerged as a new promising therapy in severe alcoholic hepatitis, as it has been shown to improve liver function and 3-month survival (Figure 2).[57]

## THERAPY FOR ALCOHOLIC CIRRHOSIS

None of the existing therapies (silymarin, SAMe, vitamin E, and pentoxifylline) improved survival in alcoholic cirrhosis other than abstinence.[14,40,41]

### Liver Transplantation

Concurrent HCV infection, smoking, and destructive drinking after liver transplant reduce survival in post-transplant patients with alcoholic cirrhosis. However, advanced alcoholic cirrhosis patients not showing significant recovery after 3 months of alcohol abstinence are unlikely to survive without transplant and should be placed on the transplant waiting list (traditional 6-month waiting period in most transplant centres). A CP score >11 in spite of at least 6 months of abstinence have improved survival with liver transplantation,[14,59] and in CP Class B cirrhosis mortality increases with transplant due to the development of different malignancies in the postoperative period.[14]

In a study, early transplantation has been shown to improve survival in patients with their first episode of alcoholic hepatitis not responding to medical therapy (6-month survival rate of 30%, with most dying within 2 months), more than transplantation following the traditional 6-month waiting period (because of the fear of relapse of drinking and that they may respond to medical therapy or abstinence).[60]

## CONCLUSION

In alcoholic liver disease, alcohol abstinence and nutritional support is of paramount importance as none of the pharmacological agents increase intermediate and long-term mortality. Alcoholic liver disease with sepsis and multi-organ dysfunction has dismal prognosis, so alcohol abuse should be diagnosed early. HCC surveillance should be done as in other causes of cirrhosis. Glucocorticoids and pentoxifylline either alone or in combination, along with other newer agents, can reduce short-term mortality but the long-term benefit is uncertain. Liver transplantation can substantially improve the survival in selected patients with alcoholic cirrhosis and severe alcoholic hepatitis that are resistant to all forms of therapy.

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# NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD): THE SEARCH FOR A CURE

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 23.11.15 **Accepted:** 18.02.16
**Citation:** EMJ. 2016;1[2]:93-100.

## ABSTRACT

An alarming rise of obesity and, along with it, non-alcoholic fatty liver disease (NAFLD), has been observed in the USA and the rest of the world. NAFLD, the most common cause of chronic liver disease in many developed countries, is not always a benign disorder and considering its growing nature, will have a serious impact on healthcare systems worldwide. The search continues for a suitable therapy for this disorder; the therapy ideally needs to be safe, effective, and affordable. The biggest hurdle in the process of developing such a therapy is our lack of a complete understanding of the pathogenesis of the disease.

Keywords: Fatty liver, non-alcoholic fatty liver disease (NAFLD), steatosis, steatohepatitis, obesity.

## INTRODUCTION

The problem of obesity has grown tremendously in the USA and the rest of the world and, along with it, several comorbidities including non-alcoholic fatty liver disease (NAFLD). NAFLD represents over 75% of chronic liver disease cases.[1] It is also one of the most common indications for liver transplantation, surpassed only by hepatitis C virus infection and alcoholic liver disease.[2] In the USA National Health and Nutrition Examination Survey, 6% of overweight and 10% of obese adolescents had an elevated serum alanine aminotransferase (ALT).[3] Studies from Japan[4,5] and England[6] reported that the prevalence of the disease has almost doubled over the last two decades and a significant increase of its occurrence in adolescents has been observed (174%).[1,7]

Although NAFLD was first described in 1980, we still lack a complete understanding of the mechanism and causes of the disease.[8] There is a pressing need for the development of non-invasive diagnostic modalities, simple screening markers, and safe, effective, and affordable therapeutic agents. The aim of this article is to discuss the current, emerging, and potential therapeutic options for the management of NAFLD.

## PATHOGENESIS AND NATURAL HISTORY

The pathogenesis of non-alcoholic steatohepatitis (NASH) is commonly described by the 'two-hit hypothesis': the first hit results from disorders of hepatic uptake, synthesis, degradation, and secretion of free fatty acids, which result in macrovesicular steatosis. This predisposes the liver to a second hit with progression from simple steatosis to steatohepatitis and cirrhosis.[9]

Fat-derived factors such as fatty acids, adiponectin, and tumour necrosis factor alpha (TNFα) modulate the hepatic inflammatory response, regulate the inflammatory response, and promote NAFLD. Adiponectin and TNFα are mutually antagonistic. Adiponectin hinders fatty acid uptake, incites fatty acid oxidation and the export of lipids, and increases hepatic insulin sensitivity. On the other hand, TNFα recruits inflammatory cells and promotes insulin resistance. The imbalance seen in patients with metabolic syndrome of increased production of TNFα associated with decreased activity of adiponectin enhances insulin resistance, resulting in fat deposition, inflammation, and cell death.[10,11]

The natural history of patients with NAFLD is poorly defined. A large cohort study has shown that the hazard ratio for general mortality and liver-related

mortality was 1.038 and 9.32, respectively.[12] The leading cause of death in patients with NAFLD is cardiovascular disease.[12] Data regarding the progression of NAFLD from simple steatosis to steatohepatitis is conflicting. In a study that included 40 patients who were followed over a median period of 11 years, there was no progression to NASH or cirrhosis, and only 30% of the patients had abnormal liver tests at the conclusion of the study.[13] Conflicting data was reported recently from a study of 52 NAFLD patients followed for 3 years. 15% had normal histology, 23% remained at baseline, 39% developed borderline NASH, and 23% developed NASH. Twenty-two of the 52 subjects had borderline NASH when the study commenced. At the conclusion of the study, 18% of the 22 patients had simple steatosis, 59% remained at borderline NASH, and 23% had NASH.[14]

The overall risk of a patient with simple steatosis progressing to cirrhosis is about 1–2%.[15] On the other hand, the risk of cirrhosis in patients with NASH varies from 0% at 5 years to 12% at 8 years.[16,17] Ekstedt et al.[18] followed 129 patients for a mean of 13.7 years and reported that 5.4% of the patients developed end-stage liver disease, including hepatocellular carcinoma (HCC). In another study of 46 patients with bridging fibrosis and 43 patients with cirrhosis, 20% developed HCC after 5 years.[19]

## CURRENT TREATMENT OPTIONS

### Lifestyle Modifications

The first-line therapy for the management of steatosis and NASH to date has been lifestyle modification focussing on dietary strategies and increased activity. However, data have been limited, and the biggest impediment is how to measure disease improvement or progression. A randomised study by Harrison et al.[20] showed that subjects who lost ≥5% of their body weight over 9 months demonstrated increased insulin sensitivity and a reduction in steatosis. In comparison, subjects who lost ≥9% of their body weight demonstrated histological improvement.

Bhat et al.[21] reported that lifestyle modification decreases insulin resistance, resulting in improvements in ALT and liver histology in patients with NAFLD. Diet and exercise have been efficacious in preventing steatosis progression to NASH.[22] Villar-Gomez at al.[23] reported that a greater extent of weight loss (induced by lifestyle changes) was associated with a greater level of improvement in NASH histological features. Weight losses of ≥10% in body weight were associated with the highest rates of NAFLD activity score reduction, NASH resolution, and fibrosis regression.[23]

There is a general consensus that heavy alcohol intake should be avoided. However, it seems that moderate use of alcohol may have a beneficial effect on NAFLD. Lower rates of steatohepatitis were reported in patients with moderate alcohol drinking compared with non-drinkers.[24] The Substance Abuse and Mental Health Services Administration (SAMHSA) defines heavy drinking as consuming ≥5 drinks on the same occasion on ≥5 of the past 30 days. On the other hand, there is general agreement about the definition of moderate drinking being no more than 3–4 standard drinks per drinking episode, and no more than 9 drinks per week for women and 12–14 per week for men.

### Dietary Supplementation

With oxidative stress being a central component to liver injury and damage, antioxidant supplementation has been extensively studied in patients with NASH. Vitamin E (an antioxidant) has been shown to reduce serum ALT levels and improve liver histology.[25] Although the randomised controlled PIVENS trial demonstrated that vitamin E can reduce inflammation and ALT levels, no improvement in fibrosis score was observed.[26,27] Consideration of risk factors must be taken into account before advocating for the use of vitamin E, as studies have suggested that increased vitamin E intake can be associated with a higher risk of developing prostate cancer,[28] as well as an increase in all-cause mortality.[29] However, more recently, published studies have reported conflicting results.[30]

Additional antioxidants that have been studied include caffeine and coffee. Both caffeine and coffee have demonstrated the ability to decrease fibrosis, steatohepatitis progression, and are associated with lower instances of NASH amongst users.[31]

While multiple other supplements, including ursodeoxycholic acid, omega-3 fatty acids, probiotics, and high-dose niacin therapy have been studied as potential treatment options for patients with NAFLD, none have produced enough evidence to justify their widespread use. In a recently published meta-analysis, probiotics were shown to lower aminotransferases, total cholesterol, and TNFα, as well as improve insulin resistance in patients with NAFLD.[32] In small studies, the efficacy of ursodeoxycholic acid in improving

liver enzymes and other measurable outcomes has been demonstrated. However, significant histologic improvement was not observed in larger studies.[33] Omega-3 fatty acids have shown promise for treating patients with NASH, with several studies utilising omega-3 supplementation demonstrating improvement in ALT levels and hepatic fat content in NASH patients.[34] High-dose niacin therapy has revealed an ability to prevent steatohepatitis in murine models.[26]

## Insulin Sensitisers

Insulin resistance plays a key role in the pathogenesis of NAFLD. Several studies of metformin have demonstrated improvement in ALT and insulin sensitivity in patients with NAFLD.[35,36] However, only a few studies have demonstrated histologic improvement.[37] Metformin was found to be less effective in decreasing liver enzymes and hepatic fat content than exercise alone.[38] In another study, metformin was reported to produce only a weak improvement compared with diet alone.[39] In a controlled study, metformin was found to have little to no histopathologic improvement in patients with insulin resistance and without diabetes.[40]

Other insulin sensitisers such as thiazolidinediones (TZDs), namely pioglitazone and rosiglitazone (pharmacologic activators of peroxisome proliferator-activated receptor gamma [PPARγ], which is known to be down regulated in models of NASH), have been studied for their efficacy in treating patients with NASH.

A randomised controlled trial (RCT) investigating rosiglitazone (FLIRT 1) reported a 31% improvement in both steatosis and transaminase levels in patients with NASH.[41] In the FLIRT 2 extension trial the results of the previous study were confirmed.[42] However, when rosiglitazone was supplemented with metformin or losartan, no improvement in histopathology was observed, compared with subjects who took rosiglitazone alone.[43]

In a study with non-diabetic NASH patients, it was determined that pioglitazone was associated with a reduction in ALT, gamma-glutamyl transferase, and ferritin.[44] The study also reported reductions in hepatocellular injury, Mallory–Denk bodies, and fibrosis. The reduction in fibrosis associated with pioglitazone therapy in NASH patients was also confirmed in a meta-analysis.[45]

A common side effect reported in all studies was weight gain in patients taking TZDs. The risk of significant cardiovascular events has led to the withdrawal of rosiglitazone from the market. Rosiglitazone had significantly higher risk of the patient developing congestive heart failure (CHF), myocardial infarction, and death when compared with pioglitazone.[46] In another study, patients treated with pioglitazone were shown to have a 0.5% higher rate of CHF, compared with the control group.[47]

## Statins

Patients with cardiovascular risk factors and disease are commonly prescribed statins as a preventative measure. The GREACE trial, a large prospective study in patients with abnormal liver enzymes and coronary artery disease, concluded that statin treatment is safe, can improve liver tests, and reduce cardiovascular morbidity in patients with mild-to-moderately abnormal liver tests, which are potentially attributable to NAFLD.[48] Data reported from the St. Francis Heart Study demonstrated a reduction in hepatic steatosis (visualised via computed tomography), in 455 patients receiving combination therapy of atorvastatin and vitamins C and E, compared with placebo.[49] However, the study did not provide any histological data.

## Surgery

According to the National Institute of Health Guidelines, bariatric surgery can be considered for individuals with a body mass index (BMI) >40 kg/m$^2$, or with a BMI of 35 kg/m$^2$ in patients with obesity-associated comorbidities who have previously tried to lose weight with diet and exercise. The presence of NASH does not seem to increase the complications of bariatric surgery.[50] Two recent meta-analyses concluded that steatosis, steatohepatitis, and fibrosis appear to improve or completely resolve in the majority of patients following bariatric surgery-induced weight loss.[51,52]

Currently, the most commonly performed bariatric procedures include Roux-en-Y gastric bypass (RYGB), laparoscopic adjustable gastric band (LAGB), and sleeve gastrectomy, whilst vertical band gastrectomy and biliopancreatic diversion are rarely performed. The majority of follow-up studies examining liver histology after RYGB surgery reported improved hepatic steatosis, inflammation, and fibrosis. However, worsening fibrosis scores were reported in four studies.[53] Taitano et al.[54] followed 160 patients after bypass surgery (median interval 20 months); a decrease in BMI from 52±10–33±8 kg/m$^2$ was observed, which was

associated with improvements in all major NASH activity scores. Resolution of fibrosis was reported in another cohort of 63 patients who underwent paired liver biopsies between the two procedures and 12 months after surgery.[55]

Thirty-six obese patients who underwent LAGB had paired liver biopsies (mean interval of 25.6 months) before and after the procedure. The weight loss following LAGB surgery was associated with a significant improvement in liver histology.[56] Significant reductions in aspartate aminotransferase (AST), ALT, triglyceride, and high-density lipoprotein (HDL) levels were reported following laparoscopic sleeve gastrectomy.[57] However, there are currently no published follow-up studies with histological data.

It is not possible to indicate bariatric surgery exclusively for NASH due to the absence of controlled evidence and long-term data.[58] Other less invasive options for bariatric therapy, such as intragastric balloons, have demonstrated benefits for liver function, insulin resistance, and histopathologic measures in obese patients with and without NASH.[59] It is imperative to realise that some patients may have undiagnosed cirrhosis, and as such may need special assessment prior to surgical intervention.

Unfortunately, patients with NAFLD can progress to end-stage liver disease, leaving liver transplantation as the only remaining treatment option. NASH-related cirrhosis is currently the third most common indication for liver transplantation, surpassed only by the hepatitis C virus and alcohol-related cirrhosis, and is anticipated to become the leading indication for liver transplantation within the next one to two decades.[60] The increasing prevalence of NAFLD in the general population also affects the presence of steatosis in deceased and live donor livers available for transplantation, which can affect the quality and the quantity of the available livers.[61] The outcome of liver transplantation in patients with NAFLD and cirrhosis due to NAFLD are comparable to those transplanted for other causes.[62] In a recent systematic review and meta-analysis report, 1, 3, and 5-year patient survival were similar in NASH and non-NASH recipients. However, cardiovascular complications and sepsis were more common as causes of death in NASH recipients.[63] NAFLD can recur after transplant or develop *de novo*. NAFLD seems to recur in at least one-third of patients who received a liver transplant due to NASH cirrhosis.[64] While NAFLD recurrence does not seem to affect overall graft and patient survival for up to 10 years, cardiovascular and infection related morbidity and mortality seem to be increased in these patients.[65]

## EMERGING AND POTENTIAL THERAPIES

The fibrates (agonists of PPARα) are used clinically for the management of dyslipidaemias. A small study (27 patients) in 2010 demonstrated a modest decrease in intrahepatic triglyceride content in obese patients taking fenofibrate compared with placebo.[66] In another report, a significant reduction in ALT associated with histologic improvement of hepatocellular ballooning degeneration was observed in patients receiving fenofibrate. However, no significant change in the amount of steatosis or fibrosis occurred.[67]

GFT505, a recently developed dual PPARα/δ agonist, has been shown to reduce hepatic and peripheral insulin sensitivity in obese patients, which is associated with a 20.5% reduction in serum ALT level.[68] In a short-term (4–8 week) Phase IIa study, GFT505 had a significant lowering effect on the concentration of liver dysfunction markers.[69] However, in a 1-year study on 274 patients, GFT505 use was not associated with significant improvement in liver fat content or fibrosis.[70] The results of an international, Phase II RCT of GFT505 in adult patients with NASH demonstrated that a daily dose of 120 mg of GFT505 induced a significant histological improvement and resolution of NASH, compared with the placebo group.[71]

Cysteamine bitartrate (RP 103) is an aminothiol antioxidant approved for the treatment of cystinosis. In a 24-week pilot, open-label, Phase IIa clinical trial in 13 children with moderate-to-severe NAFLD, seven patients demonstrated a reduction in liver enzymes in the absence of a significant change in mean BMI. Mean cytokeratin-18 (CK-18) fragment levels decreased by 43% from baseline, while plasma adiponectin levels increased by 31%.[72]

Obeticholic acid (OCA) is a farnesoid X receptor agonist which has been shown to increase insulin sensitivity and exert anti-inflammatory and antifibrotic effects in preclinical models. A RCT (FLINT trial) of 282 subjects with NASH demonstrated a significantly improved efficacy of OCA relative to placebo in terms of reductions in AST and ALT. A total of 45% of 110 patients in the OCA group demonstrated an improvement in histological features. However, the change did

not reach statistical significance. Compared with placebo, treatment with OCA was also associated with higher levels of total serum cholesterol and low-density lipoprotein cholesterol, and a decrease in HDL cholesterol.[73]

Simtuzumab is a humanised monoclonal antibody against lysyl oxidase-like 2, an enzyme that is vital to the biogenesis of connective tissue. Simtuzumab is currently in the process of development as an antifibrotic agent. In a study of 20 patients with liver fibrosis of various aetiologies, improvement in transaminases was observed, suggesting a possible anti-inflammatory effect in addition to the antifibrotic effect.[74] A Phase IIb trial in patients with NASH and compensated cirrhosis is currently underway.

Aramchol is an inhibitor of the activity of stearoyl-coenzyme A desaturase 1 in the liver. The inhibition may result in a decrease in storage triglycerides and other esters of fatty acids. In a RCT of 60 patients with biopsy-confirmed NAFLD (including six subjects with NASH), patients on aramchol had their liver fat content decreased by 12.57–22.14%.[75] There is an ongoing Phase II trial in overweight/obese patients with prediabetes or Type 2 diabetes mellitus and NASH.

Liraglutide is a long-acting glucagon-like peptide-1 receptor agonist approved for the treatment of diabetes and the treatment of obesity in adults with related comorbidity. Liraglutide was reported to be safe and well tolerated. Improvement of liver enzymes was reported in a 26-week trial in patients with diabetes and elevated liver enzymes.[76]

Emricasan is a potent irreversible pan-caspase inhibitor. Caspases play a core role in the processes of apoptosis, inflammation, and activation of cytokines such as interleukin (IL)-1β and IL-18. All these processes are known to play important roles in the pathogenesis of NAFLD. In a recent 28-day Phase II study in patients with NAFLD and elevated ALT, treatment with emricasan led to statistically significant reductions in ALT and CK-18.[77]

Cenicriviroc is an immunomodulator and dual inhibitor of chemokine receptors CCR2 and CCR5. In patients with HIV, treatment with cenicriviroc was associated with improvements in AST to platelet ratio and fibrosis-4 (FIB-4) scores; correlations were observed between changes in AST to platelet ratio and FIB-4 scores and soluble CD14 levels at Week 48.[78] Phase II studies in patients with NAFLD are underway.

Remogliflozin has been shown to improve insulin sensitivity in subjects with Type 2 diabetes through SGLT2 inhibition. Treatment with remogliflozin in a 12-week trial in diabetic patients resulted in a 40% reduction in ALT levels in subjects with elevated values at baseline.[79]

Two novel classes of potential pharmacotherapies are the glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors, collectively known as incretin-based therapies, which have several metabolic and anti-inflammatory actions that may be of benefit in NAFLD and Type 2 diabetes mellitus. Treatment with incretin was found to significantly reduce steatosis, inflammation, and fibrosis.[80]

The use of pentoxifylline (anti-TNFα) therapy in NAFLD has also been investigated. Pentoxifylline combined with fenofibrate resulted in beneficial effects on direct and indirect markers of liver fibrosis, liver stiffness, and insulin resistance.[81]

## NOVEL MOLECULAR TARGETS

In the near future, the identification of key molecular targets that play an important role in the pathogenesis of NAFLD may lead to the development of effective therapeutic options (Table 1).

MCC950, a potent and selective NLRP3 inflammasome inhibitor, has been shown to prevent or reverse inflammation, injury, and fibrosis in two different murine models of NASH. Targeting NLRP3 may be a logical new direction in NASH pharmacotherapy.[82]

Palmitate (PA), a lipotoxic free fatty acid, is implicated in hepatocyte apoptosis, macrophage-mediated liver inflammation, and activation of the IRE1α branch of the endoplasmic reticulum stress response. PA-induced extracellular vesicles stimulate macrophage chemotaxis and this may be a mechanism for the recruitment of macrophages to the liver under lipotoxic conditions. It is possible that interference with this macrophage recruitment response may be a therapeutic avenue in NASH.[83]

NAFLD and obesity are characterised by altered gut microbiota, inflammation, and gut barrier dysfunction. Mucin-2 (Muc2) is the major component of the intestinal mucus layer. It has recently been shown that an impaired gut barrier in Muc2 deficient mice elicits a strong intestinal immune response that is associated with the release of IL-22.

**Table 1: Novel therapeutic agents aiming at key molecular targets.**

| |
|---|
| **MCC950:** Potent and selective NLRP3 inflammasome inhibitor |
| **Palmitate:** Lipotoxic free fatty acid |
| **INT-767:** Dual agonist of the nuclear receptor farnesoid X receptor and the G-protein coupled receptor |
| **DRX-065:** stabilised deuterated R-enantiomer of pioglitazone |

Increased systemic IL-22 may mediate beneficial metabolic and anti-inflammatory effects in patients with NAFLD.[84]

INT-767, a dual agonist of the nuclear receptor farnesoid X receptor (FXR) and the G-protein coupled receptor, TGR5. The dual FXR-TGR5 agonist INT-767 has been reported to be able to markedly and significantly arrest and reverse progression of liver disease in mice, even when treatment is started in the presence of obesity, insulin resistance, and NASH.[85]

DRX-065 is a stabilised deuterated R-enantiomer of pioglitazone. DRX-065 has pharmacological properties desirable for the treatment of NASH (mitochondrial function modulation, non-steroidal anti-inflammatory effects, and glucose lowering effects) without the undesired PPARγ-related weight gain side effects. Therefore, DRX-065 may represent a potentially significant therapeutic improvement over pioglitazone, a drug already recommended off-label for the treatment of NASH.[86]

## CONCLUSION

The problem of obesity has grown tremendously over the last three decades, gradually transforming into an epidemic.[87] NAFLD has become one of the major diseases plaguing the USA, as well as the rest of the world. The search continues for safe, effective, and affordable therapy for this disorder. The biggest hurdle in the process of developing the ideal therapy is our incomplete understanding of the pathogenesis of the disease. Several therapeutic agents have surfaced over the last few years and though all of these promising agents are still in their infancy, with further research they may become effective therapeutic options. An ounce of prevention is worth a ton of cure. Increased awareness is crucial through public education focussing on lifestyle modification strategies, especially in high-risk groups. Until we reach our goals, nothing is available for NAFLD.


## Acknowledgements

I am grateful to Dr. William Balistreri for suggesting the title.


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# PRIMARY PARAGANGLIOMA OF THE THYROID GLAND: CLINICAL AND IMMUNOHISTOLOGICAL ANALYSIS WITH A LITERATURE REVIEW

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 14.12.2015 **Accepted:** 23.02.2016
**Citation:** EMJ. 2016;1[2]:101-106.

## ABSTRACT

Primary paraganglioma of the thyroid is a rare neuroendocrine tumour, often mistaken for other thyroid neoplasms. Here, we describe a case of initially misdiagnosed primary paraganglioma of the thyroid and study its clinical presentation, management, investigation, and immunohistological findings.

A 72-year-old male presented with a left-sided solitary thyroid lobe and isthmus nodule. Ultrasound, fine needle aspiration, and computed tomography did not provide a clear diagnosis and subsequently, a left lobectomy and isthmusectomy were performed. The initial histopathological findings of the tumour revealed positivity to chromogranin and calcitonin, suggesting a medullary carcinoma replacing the left lobe of the thyroid. In a second histopathological review at an external laboratory, the tumour cells showed positive focal staining for chromogranin, but were negative for both calcitonin and monoclonal carcinoembryonic antigen, suggesting thyroid paraganglioma. This case highlights the importance of accurate histopathological diagnosis and the need to be aware of the possibility of thyroid paraganglioma initially presenting as a thyroid nodule.

Keywords: Primary paraganglioma, thyroid gland, clinical immunohistological analysis, medullary carcinoma, hyalinising adenoma.

## INTRODUCTION

Paragangliomas are neuroendocrine tumours that originate from the neural crest paraganglia of the autonomic nervous system.[1] The tumours can develop in a multitude of sites, including the head, neck, thorax, and abdomen. Within the head and neck region, the growths can present in the carotid, jugulotympanic, laryngeal, vagal, and orbital bodies. However, the thyroid gland represents an uncommon site for paraganglioma cells, with primary paraganglioma of the thyroid accounting for <0.1% of thyroid neoplasms.[2] In many cases, these tumours are endocrinologically silent.[3]

Due to the rarity of these tumours, they are often misdiagnosed, which could result in inappropriate management and treatment. Thus, proper diagnosis both clinically and histologically is of great importance. We will describe a case of misdiagnosed primary paraganglioma of the thyroid, which was initially thought to be a medullary carcinoma. In this article we will discuss the initial presentation of the case, its management, and how the final diagnosis was suggested histologically.

### Case Report

A 72-year-old male presented to the hospital with dyspnoea and hoarseness of voice caused by a left-sided solitary thyroid lobe and isthmus nodule. Physical examination of the patient revealed a large, solid, firm, painless mass on the left thyroid lobe without any palpable cervical lymph nodes.

The right lobe was unremarkable. The patient's vitals were within normal range. Indirect laryngoscopy showed paralysis of the left vocal fold, though movement in the right vocal fold was normal. Serum thyroid-stimulating hormone, triiodothyronine (T3), free thyroxin (T4), calcitonin, thyroglobulin, cortisol, urine metanephrines, and adrenocorticotropic hormone were all within normal limits. There was, however, elevated C-reactive protein levels (78.3 mg/L [<5.6 mg/L]) and an elevated neutrophil count 84.2% (42.0–75.0%).

The patient's family history was free of thyroid disease. An aneurysm of the abdominal aorta measuring 5.1 cm in diameter was noted in his personal medical history. There was no hypertension, flushing, diarrhoea, or other symptoms related to catecholamine hypersecretion present.

Ultrasound revealed a 7x4.3 cm mass showing microcystic features internally and extending inferiorly until the upper mediastinum (T3 vertebral level). Oedema was seen in subcutaneous fat, which could be indicative of inflammation. In addition, small circular lymph nodes were seen; the largest of which measured 1.2 cm in diameter. Ultrasound guided fine needle aspiration (FNA) biopsy of the thyroid was performed. The cytology report revealed erythrocytes, rare leukocytes, single follicular cells, and small follicular type aggregates without cytological abnormalities, which showed some oxyphilic (stainable without acid dye) metaplastic forms and were suggestive of a follicular lesion. Following FNA, computed tomography (CT) of the cervical area revealed that the left nodule was causing pressure posteriorly, displacing the left common carotid artery and outwardly displacing the internal jugular vein (Figure 1). Diagnostics did not include genetic testing, or a fluorodeoxyglucose-positron emission tomography scan to look for other tumours. The case was reviewed in a multidisciplinary team setting where it was decided that treatment should proceed with surgical resection of the left lobe and isthmus.

Surgery proved very difficult, due to the presence of a firm neoplasm that spread outside the thyroid capsule and was firmly attached to the surrounding neck structures, in particular to the left recurrent laryngeal nerve, trachea, and oesophagus. No lymph node enlargement was present. Intra-operative neuromonitoring was used, but there was no signal response from stimulating the left laryngeal recurrent nerve or the left vagus nerve. There were no post-operative complications and the patient left hospital 4 days following surgery.

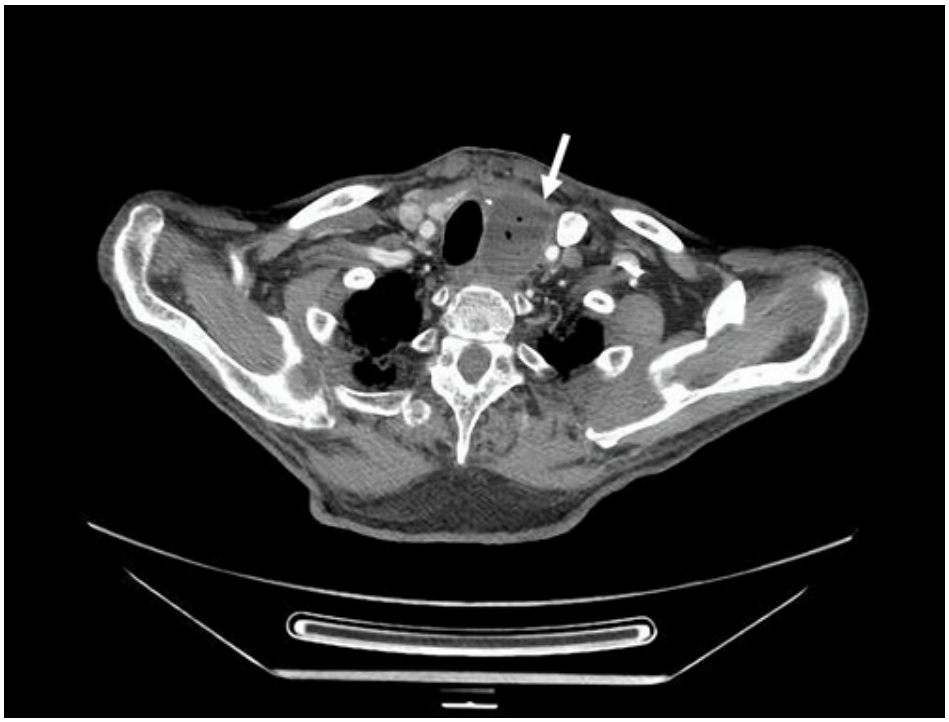

**Figure 1: Computed tomography of the cervical spine revealing the left nodule pushing and causing pressure, posteriorly displacing the left common carotid artery and outwardly displacing the internal jugular vein.**

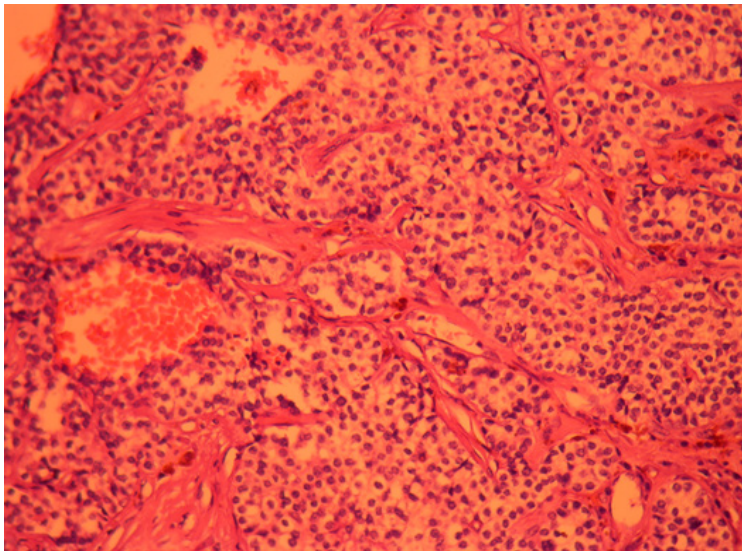

**Figure 2: Thyroid tissue infiltrated by a tumour that is composed of closely packed solid groups of polygonal neoplastic cells with eosinophilic cytoplasm and round-to-oval nuclei containing granular chromatin. The groups of cells are separated by abundant capillaries and dilated vessels.**

### Immunohistology

Conventional histology and immunochemistry were performed. On microscopic examination the left lobe was extensively infiltrated by a small-celled tumour. The tumour was composed of fused small malignant cells infiltrating the fibrous stroma and replacing the entire left lobe. No obvious amyloid was identified (Figure 2).

The immunomarkers used included synaptophysin, chromogranin A, calcitonin, thyroglobulin, thyroid transcription factor 1 (TTF1), cytokeratin (CK)-7, and CK20. The neoplastic cells were positive for chromogranin and calcitonin, which suggested a diagnosis of medullary carcinoma replacing the left lobe of thyroid. Due to the lack of experience within the local lab in identifying rare types of cancer histology, the slides were later sent to London, UK, for a re-assessment of the histopathology and immunochemistry. Upon re-examination, histology revealed thyroid tissue infiltrating with a tumour composed of closely packed solid groups of polygonal neoplastic cells with pale stained cytoplasm and rounded darkly stained nuclei. Immunology showed positive focal staining for chromogranin and negative for both calcitonin and monoclonal carcinoembryonic antigen (CEA). The conclusion was reached that although calcitonin-poor medullary carcinoma has been described, the additional absence of CEA suggested that thyroid paraganglioma should be considered a stronger possibility.

## Outcome and Follow-up

One year after diagnosis, the patient is alive and well without evidence of disease recurrence.

## DISCUSSION

Paraganglioma of the thyroid gland is an extremely rare neuroendocrine tumour[4] first described in 1964. To the best of our knowledge, between 1964 and 2013 a total of 39 cases were reported in the literature. Of the reported cases, only five patients were male (male:female, 1:6.8), with an age range of 9–73 years old at presentation (median, 50 years old; mean, 47.8 years old; Table 1). The location of the paragangliomas reported varies with respect to the thyroid gland as the tumour can arise at the isthmus, or in the left and/or right thyroid gland tissues. In most cases, paragangliomas are confined to the thyroid gland; however, some may be locally invasive to the tracheal wall,[5,6,7] larynx/pharynx,[7] cricoid cartilage,[1] subglottis,[8] or recurrent laryngeal nerve.[9]

Clinically, paraganglioma of the thyroid gland can mimic other more common thyroid pathologies and presents as a mass or nodule. As a result, the tumour may go unnoticed, or be dismissed as a goitre, thyroglossal cyst, or follicular adenoma for a significant amount of time. In some cases, the tumour can also present with additional pathologies such as papillary thyroid carcinoma, parathyroid adenoma, and bilateral carotid body

paragangliomas.[14] What makes the diagnosis more challenging is its immunohistology, which may be difficult and is often mistaken for medullary carcinoma or hyalinising adenoma of the thyroid. In our case, the paraganglioma was initially thought to be a medullary carcinoma. This confusion can arise due to medullary carcinoma exhibiting a nesting (paraganglioma-like) pattern of growth.

**Table 1: Primary paraganglioma of thyroid gland; review of 39 cases reported in literature.**

| Author | Sex | Age (Years) | Presenting symptom | Surgery and/or other treatment | Follow-up |
|---|---|---|---|---|---|
| Armstrong[5] | F | 67 | Mass | Left lobectomy, isthmusectomy, tracheal resection of rings 3–6 | Dead (colon adenocarcinoma) 8 years post surgery |
| | M | 64 | Mass, dyspnoea on exertion, progressing stridor | Total thyroidectomy, tracheal resection | Alive and well 14 years post surgery |
| | M | 60 | Right mass | Total thyroidectomy | - |
| Ashraf[10] | F | 40 | Right mass | Surgical resection | - |
| Banner[11] | F | 36 | Mass | Left lobectomy | - |
| Bizollon[12] | F | 48 | Cold nodule | - | - |
| Brownlee[8] | F | 27 | Mass | Right lobectomy, right subglottic laryngectomy | Alive and well 18 months post surgery |
| Buss[13] | F | 50 | Tender cold nodule | Left hemithyroidectomy | Alive and well 30 months post surgery |
| Cayot[14] | F | 58 | Enlarging goitre | Total thyroidectomy | - |
| Corrado[15] | F | 46 | Mass, hypertension | Right lobectomy, isthmusectomy | - |
| de Vries[16] | F | 73 | Mass, hoarseness, trachea compression | Left hemithyroidectomy | Alive and well 2 years post surgery |
| Erem[17] | F | 58 | Multinodular goitre, growth | Right lobectomy, isthmusectomy, partial left lobectomy | Alive and well 3 months post surgery |
| Ferri[9] | F | 63 | Mass, hypertension | Right lobectomy | Alive and well 18 months post surgery |
| Foppiani[18] | F | 51 | Hot nodule, hyperthyroidism | Total thyroidectomy | Alive and well 5 years post surgery |
| Gonzalez[19] | F | 36 | Mass | Total thyroidectomy | Alive and well 2 months post surgery |
| Haegert[2] | F | 36 | Tender cold nodule | Left hemithyroidectomy | Alive and well 5 years post surgery |
| Hughes[20] | F | 50 | Cold nodule | Total thyroidectomy | Alive and well 2 years post surgery |
| Kronz[21] | M | 55 | Hypertension, left lesion | Left lobectomy, isthmusectomy | Alive and well 9 months post surgery |
| | F | 52 | Enlarging neck mass, trachea compression | Total thyroidectomy, radiotherapy | Alive and well 6 years post surgery |
| La Guette[22] | F | 55 | Mass | Total thyroidectomy | Alive and well 4 years post surgery |
| | F | 64 | Cold nodule | Left hemithyroidectomy | Alive and well 7 years post surgery |
| | F | 56 | Mass | Right hemithyroidectomy | Alive and well 8 years post surgery |
| Massaioli[23] | F | 9 | Mass | Subtotal thyroidectomy | Alive and well 5 months post surgery |
| Mitsudo[6] | F | 50 | Mass, hypertension | Total thyroidectomy, segmental anterior resection of trachea | Alive and well 2 years post surgery |
| Mun[24] | F | 40 | Recurrent multinodular goitre | Total thyroidectomy | Alive and well |

**Table 1 continued.**

| Author | Sex | Age (Years) | Presenting symptom | Surgery and/or other treatment | Follow-up |
|---|---|---|---|---|---|
| Napolitano[25] | F | 47 | - | Total thyroidectomy | Alive and well 6 months post surgery |
| Olofsson[7] | F | 44 | Mass | Left lobectomy, partial pharyngectomy, total laryngectomy, partial tracheal resection | Alive and well 7 years post surgery |
| Phitayakorn[26] | F | 41 | Mass | Surgical resection | Alive and well 14 months post surgery |
| | F | 73 | Enlarging right hypervascular mass | Right hemithyroidectomy | Alive and well 13 months post surgery |
| Skiadas[27] | F | 54 | Mild tachycardia, hypertension, cold nodule | Total thyroidectomy | Alive and well 22 months post surgery |
| Tiong[28] | F | 52 | Mass | Left lobectomy | Alive and well 2 years post surgery |
| Van Miert[4] | F | 63 | Mass, hypertension | Radiotherapy | - |
| Vera-Cruz[29] | F | 32 | Mass | Right hemithyroidectomy | Alive and well 4 years post surgery |
| Vodovnik[30] | F | 46 | Palpable tender mass, hypertension | Right lobectomy | - |
| Yano[31] | M | 24 | Right hypervascular mass | Right lobectomy | Alive and well 6 months post surgery |
| Yu[32] | F | 30 | Mass | Left lobectomy, subtotal right lobectomy | Alive and well 39 months post surgery |
| | M | 47 | Right mass | Right lobectomy | Alive and well 47 months post surgery |
| | F | 37 | Mass | Right lobectomy, isthmusectomy, partial left lobectomy | Alive and well 10 months post surgery |
| Zantour[1] | F | 32 | Mass | Total thyroidectomy, resection of cricoid cartilage | Alive and well 6 years post surgery |

M: Male; F: Female.

The diagnosis of paraganglioma of the thyroid is difficult to confirm using only FNA, ultrasound, or CT. Diagnosis is usually confirmed post surgically with immunohistology of the resected mass. Immunohistology helps distinguish medullary carcinoma and hyalinising adenoma of the thyroid from paraganglioma of the thyroid. It is often difficult to distinguish between medullary carcinoma and paraganglioma of the thyroid gland, as both have clusters of cells with granular cytoplasm (chromogranin A, synaptophysin, and neuron-specific enolase positive) and a richly vascularised stroma. However, only paraganglioma of the thyroid gland exhibits S-100 staining and lacks CK, CEA, and calcitonin staining.[9] Conversely, some medullary carcinomas stain S-100.[15,22] As the clinical management and sequel strategy of each respectively vary, it is vital to differentiate between them. Medullary carcinomas originate in the parafollicular C cells of the thyroid and thus stain with calcitonin. Additionally, they contain amyloid material and thus can be stained with Congo red. Rarely, cases of medullary carcinoma may be negative for calcitonin,[15] and paraganglioma may be positive for calcitonin or CK,[21] which might make differentiating between the two tumours even more challenging.

Another neoplasm that can sometimes be confused as paraganglioma is hyalinising adenoma of the thyroid. Hyalinising adenoma is a thyroid tumour of follicular cell origin with a trabecular pattern consisting of cells arranged around delicate vessels. Hyalinising adenoma stains positive for

thyroglobulin and TTF1. However, like paraganglioma of the thyroid, it stains negative for calcitonin and it may stain positive for chromogranin A and neuron-specific enolase.[33] This may also make the diagnosis of paraganglioma more challenging.

The majority of reported patients are alive and well with no evidence of disease recurrence following resection of a paraganglioma of the thyroid gland. In most cases, surgical resection and long-term follow-up were the preferred management options. One case received supplemental radiotherapy,[21] and another radiotherapy alone.[4] Radiotherapy is recommended where there is a suspicion of residual tumour, or when surgery is not feasible.

In summary, preoperative examinations are not ideal for diagnosis of paraganglioma. With the correct immunohistological techniques, it is possible to confirm paraganglioma of the thyroid gland and to rule out other pathologies, despite the variability in immunohistological staining between different neoplasms. Paraganglioma of the thyroid gland appears to have a favourable outcome, despite its challenging diagnosis; surgery and long-term follow-up is the advised management plan.

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# OUTCOMES OF SALVAGE LYMPH NODE DISSECTION FOR PROSTATE CANCER WITH CLINICAL NODAL RELAPSE: RESULTS OF A MULTICENTRIC, RETROSPECTIVE STUDY

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**Disclosure:** The authors have declared no conflicts of interest.
**Received:** 05.01.16 **Accepted:** 15.02.16
**Citation:** EMJ. 2016;1[2]:108-115.

## ABSTRACT

Introduction: Salvage lymph node dissection (sLND) is a treatment option for prostate cancer (PCa) patients with nodal recurrence after radical therapy to delay tumour progression and hormonal treatment. We evaluated the outcomes in terms of biochemical recurrence (BCR), clinical regression, and cancer specific survival (CSS) in a large, multicentric series of patients treated with sLND for nodal recurrence of PCa.

Methods: We retrospectively reviewed the records of 106 consecutive patients with BCR of PCa after radical treatment who underwent sLND between 2007 and 2013 at three tertiary centres. BCR was defined as prostate-specific antigen (PSA) >0.2 ng/mL. Clinical recurrence (CR) was defined as a positive imaging study or biopsy for metastasis after sLND. Kaplan–Meier curves calculated BCR-free survival (BFS), CR-free survival (CRS), and CSS. Cox regression analyses were performed to identify predictors of CR.

Results: Median number of nodes removed at sLND was 21.7, with a median of three positive nodes. Immediate biochemical response after surgery was achieved in 50.9% of patients. At a median follow-up of 22.5 months, biochemical failure and CR were experienced by 67.9% and 40.5% of patients, respectively. At 2 years, BFS, CRS, and CSS were 25%, 52%, and 92%, respectively. Castrate-resistant prostate cancer (CRPC) status, PSA level at sLND, and presence of biochemical failure after sLND were significantly associated with CR after surgery.

Conclusions: sLND represents a valid treatment option for selected patients with nodal recurrences, achieving a CR-free status in more than half of patients at 2 years. Patients with CRPC status or high PSA values might not be the best candidates for a sLND.

Keywords: Salvage lymph node dissection (sLND), prostate cancer (PCa), biochemical recurrence (BCR), choline positron emission tomography (PET).

## INTRODUCTION

Although radical treatments for localised prostate cancer (PCa) achieve excellent cancer control rates,[1] around 40% of patients develop a biochemical recurrence (BCR),[1,2] which can be associated with local or systemic recurrence of PCa. These individuals, who are at higher risk of death from PCa,[3] can have nodal metastases as the only sites of recurrent disease.[4] Traditionally, such patients would be considered as harbouring systemic disease, and thus be managed with hormone replacement therapy (HRT).[5] Recent evidence, however, supports the effectiveness of salvage lymph node dissection (sLND) as a treatment option to delay tumour progression and thus postpone

HRT in patients with disease relapse limited to lymph nodes (LN).[6] In this light, $^{11}$C-choline positron emission tomography (PET)/computed tomography (CT) currently plays an essential role in the early detection of nodal metastases to correctly select patients suitable for sLND.[7,8]

Previous studies have reported that although most patients inevitably progress to BCR after sLND, roughly 40% do not experience any further clinical recurrence (CR) even at long-term follow-up.[9,10] These are promising results; however, the feasibility of sLND in clinical practice remains limited by the lack of data concerning oncologic and surgical outcomes of this pioneering surgery. The aim of our study is to report the outcomes in terms of BCR, CR, and cancer-specific survival (CSS) in a multicentric series of patients treated with sLND for nodal recurrence of PCa. To our knowledge, this is the largest series published to date.

## METHODS

### Patient Population

After institutional review board approval, we retrospectively reviewed the records of 106 consecutive patients with BCR of PCa after radical treatment (radical prostatectomy [RP], N=102; external beam radiation therapy [EBRT], N=3; brachytherapy, N=2) who underwent $^{11}$C-choline PET/CT and sLND between 2007 and 2013 at three tertiary centres. BCR was considered to be a rise of prostate-specific antigen (PSA) to >0.2 ng/mL after RP and a PSA level >2 ng/mL higher than the PSA nadir value after radiotherapy.[11] Castrate-resistant prostate cancer (CRPC) patients were defined as patients with <1.7 nmol/L serum testosterone and biochemical progression.[5] All but six patients (who only had findings of enlarged nodes in CT scans) showed pathological uptake in at least one LN in PET/CT imaging. Pelvic and/or retroperitoneal sLND was performed according to the location of positive nodes at imaging. Patients referred for sLND had BCR after radical treatment for PCa, with evidence of nodal disease only at imaging. Preoperative imaging modalities were as previously described.[8]

### Surgical Procedure and Follow-Up

Surgical dissection was not restricted to the PET/CT targeted area, but was extended to neighbouring regions according to surgical preference. Given the current unavailability of a recommended surgical template, no standardised surgical approach could be adopted. An open approach was used in all but two patients, in whom laparoscopic sLND was performed. After sLND, surgical specimens were processed according to standard pathology protocols and evaluated by a dedicated uro-pathologist in each institution. Use of any therapy after surgery, including HRT, was decided on a case-by-case basis following multidisciplinary consultation. Follow-up consisted of periodical PSA testing and clinical visits. Postoperative imaging, including CT, magnetic resonance imaging, bone scintigraphy, or $^{11}$C-choline PET/CT was performed in cases of BCR after sLND. Complications were reported according to the modified Clavien–Dindo classification.[12]

### Statistical Analysis

Descriptive statistics were used to explore perioperative and pathologic variables. Primary outcomes were time to biochemical failure, CR, and cancer-specific mortality. Kaplan–Meier survival curves and life tables were calculated for each outcome. Biochemical failure comprised both patients with BCR after the initial PSA response (<0.2 ng/mL) following sLND, and those not attaining a PSA <0.2 ng/mL (immediate BCR). Biochemical response (BR) included patients attaining a PSA <0.2 ng/mL. CR was defined as a positive imaging study or biopsy for metastasis after sLND. Univariable and multivariable Cox regression analyses were performed to identify predictors of CR. Statistical significance was claimed for $p<0.05$. All statistical analyses were performed with SPSS v.20 (IBM, New York, USA).

## RESULTS

### Baseline Characteristics and Pathologic Outcomes

Table 1 depicts the baseline characteristics of all patients included in the study, both at the time of primary treatment and at sLND. At sLND, median patient age was 65 years (range, 48–81), with a median PSA of 3.1 ng/mL (range, 0.2–47). Mean time from PET/CT to sLND was 1.5 months (SD±0.7). Overall, PET/CT detected a median of 1.5 positive nodes per patient. Data concerning PET/CT accuracy were previously reported.[8] Adjuvant and salvage therapies before sLND were adopted in a large number of patients. Nineteen patients (18%) already possessed CRPC status before undergoing sLND. The median number of nodes removed was 21.7 (range, 2–78), with a

median of three positive nodes on final histology (range, 0–33). sLND found no positive nodes in 16 patients (15%).

## Patient Outcomes, Survival, and Complications

Surgical outcomes are shown in Table 2. BR immediately after surgery was achieved by 50.9% of patients. At last follow-up, biochemical failure was noted in 67.9% of cases, with a mean time of 19 months. CR was experienced by 40.5% of patients, mainly at nodal or bone level, with a mean time of 38 months. At a median follow-up of 22.5 months, only five patients of our series died from PCa. Kaplan–Meier survival curves for BCR-free survival (BFS), CR-free survival (CRS), and CSS are shown in Figure 1. At 2 years, BFS, CRS, and CSS were 25%, 52%, and 92%, respectively. Thirty-six patients (33.9%) experienced postoperative complications, of which only 10 (9.4%) were graded as Clavien III-IV (Table 2).

## Univariable and Multivariable Cox Regression Models Predicting Clinical Progression

In univariable Cox regression models considering the main preoperative and postoperative variables, CRPC status, PSA level at sLND, and presence of biochemical failure after sLND were significantly associated with CR after surgery (Table 3). In multivariable analyses, all of these variables represented independent predictors of CR (all $p<0.02$).

**Table 1: Baseline patient characteristics.**

| Variable | All patients (N=106) | Radical prostatectomy (N=101) | Radiation therapy or brachytherapy (N=5) |
|---|---|---|---|
| **Patient characteristics at time of primary treatment** | | | |
| Age (years), mean±SD<br>Age (years), median (range) | 59.4±6.8<br>59 (46–75) | 59.5±6.8<br>59 (46–75) | 57.6±5.4<br>55 (53–66) |
| Years considered | 1993–2013 | 1993–2013 | 2001–2011 |
| PSA, ng/mL, mean±SD<br>PSA, ng/mL, median (range) | 11.0±8.4<br>8.6 (2.0–44.6) | 11.0±8.1<br>8.7 (2.5–44.6) | 12.0±13.1<br>4.6 (2.0–33.4) |
| GS<br>• ≤6, n (%)<br>• 7, n (%)<br>• 8–10, n (%) | <br>4 (3.8)<br>60 (56.6)<br>42 (39.6) | Pathologic GS<br>3 (3.0)<br>58 (57.4)<br>40 (39.6) | Bioptic GS<br>1 (20.0)<br>2 (40.0)<br>2 (40.0) |
| Stage at radical prostatectomy<br>• pT3, n (%)<br>• pNx, n (%)<br>• pN1, n (%) | <br>NA<br>NA<br>NA | Pathologic stage<br>65 (64.3)<br>23 (22.7)<br>7 (6.9) | Clinical stage<br>1 (20)<br>NA<br>NA |
| Positive margins, n (%) | NA | 41 (45.1) | NA |
| Nodes removed, mean (range) | NA | 11.7 (2–45) | NA |
| Positive nodes, mean (range) | NA | 1.75 (1–4) | NA |
| Adjuvant therapies, n (%)<br>• RT<br>• HRT | <br>22 (20.7)<br>11 (10.3) | <br>22 (21.3)<br>10 (9.7) | <br>NA<br>1 (20) |
| Months to BCR, mean±SD<br>Months to BCR, median (range) | 40.4±37.4<br>31.0 (1–190) | 38.0±34.3<br>30.5 (1–190) | 81.8±66.3<br>99.0 (12–150) |
| **Patient characteristics at time of sLND** | | | |
| Age (years), mean±SD<br>Age (years), median (range) | 65.3±6.9<br>65.5 (48–81) | 64.3±7.0<br>65 (48–81) | 66.2±6.7<br>67 (56–75) |
| Years considered | 2007–2014 | 2007–2014 | 2013–2014 |
| Time from primary treatment to sLND (months), mean±SD<br>Time from primary treatment to sLND (months), median (range) | 70.2±49.4<br>59 (6–233) | 68.7±48.8<br>56 (6–233) | 101.8±55.6<br>106 (20–159) |
| Salvage therapies before sLND, n (%)<br>• RT<br>• HRT<br>• Chemotherapy + HRT | <br>53 (50)<br>22 (20.7)<br>8 (7.5) | <br>53 (52.4)<br>20 (19.8)<br>8 (7.9) | <br>NA<br>2 (40)<br>0 (0) |

**Table 1 continued.**

| Variable | All patients (N=106) | Radical prostatectomy (N=101) | Radiation therapy or brachytherapy (N=5) |
|---|---|---|---|
| PSA, ng/mL, mean±SD<br>PSA, ng/mL, median (range) | 3.1±5.0<br>1.0 (0.2–47) | 2.1±3.1<br>1.0 (0.1–17) | 2.5±1.7<br>1.4 (1.3–4.8) |
| CRPC, n (%) | 19 (17.8) | 16 (15.7) | 3 (60) |
| Positive nodes at PET/CT, mean±SD<br>Positive nodes at PET/CT, median (range) | 2.01±1.6<br>1.5 (0–9) | 1.99±1.6<br>1 (0–9) | 2.4±1.1<br>2 (1–4) |
| Positive locations at PET/CT, n (%)<br>• Pelvic only<br>• Retroperitoneal only<br>• Pelvic plus retroperitoneal<br>• Negative | <br>82 (77.3)<br>6 (5.6)<br>12 (11.3)<br>6 (5.6) | <br>78 (77.2)<br>5 (4.9)<br>12 (11.8)<br>6 (5.9) | <br>4 (80)<br>1 (20)<br>0 (0)<br>0 (0) |
| Nodes removed at sLND, mean±SD<br>Nodes removed at sLND, median (range) | 21.7±14.5<br>20.5 (2–78) | 20.5±12.8<br>18.0 (2–62) | 47.6±23.3<br>45 (24–78) |
| Positive nodes at sLND, mean±SD<br>Positive nodes at sLND, median (range) | 4.7±5.4<br>3 (0–33) | 4.4±4.5<br>3 (0–20) | 10.8±14.4<br>3 (0–33) |
| Positive locations at sLND, n (%)<br>• Pelvic only<br>• Retroperitoneal only<br>• Pelvic plus retroperitoneal<br>• Negative | <br>66 (62.2)<br>5 (4.7)<br>19 (17.9)<br>16 (15.1) | <br>65 (64.3)<br>4 (3.9)<br>19 (18.8)<br>14 (13.8) | <br>1 (20)<br>1 (20)<br>0 (0)<br>2 (40) |

sLND: salvage lymph node dissection; PET: positron emission tomography; CT: computed tomography; GS: Gleason score; pT3: tumour extends beyond the prostate; pNx: regional lymph nodes were not assessed; pN1: regional lymph nodes affected by tumour; BCR: biochemical recurrence; RT: radiotherapy; HRT: hormone replacement therapy; PSA: prostate-specific antigen; CRPC: castrate-resistant prostate cancer.

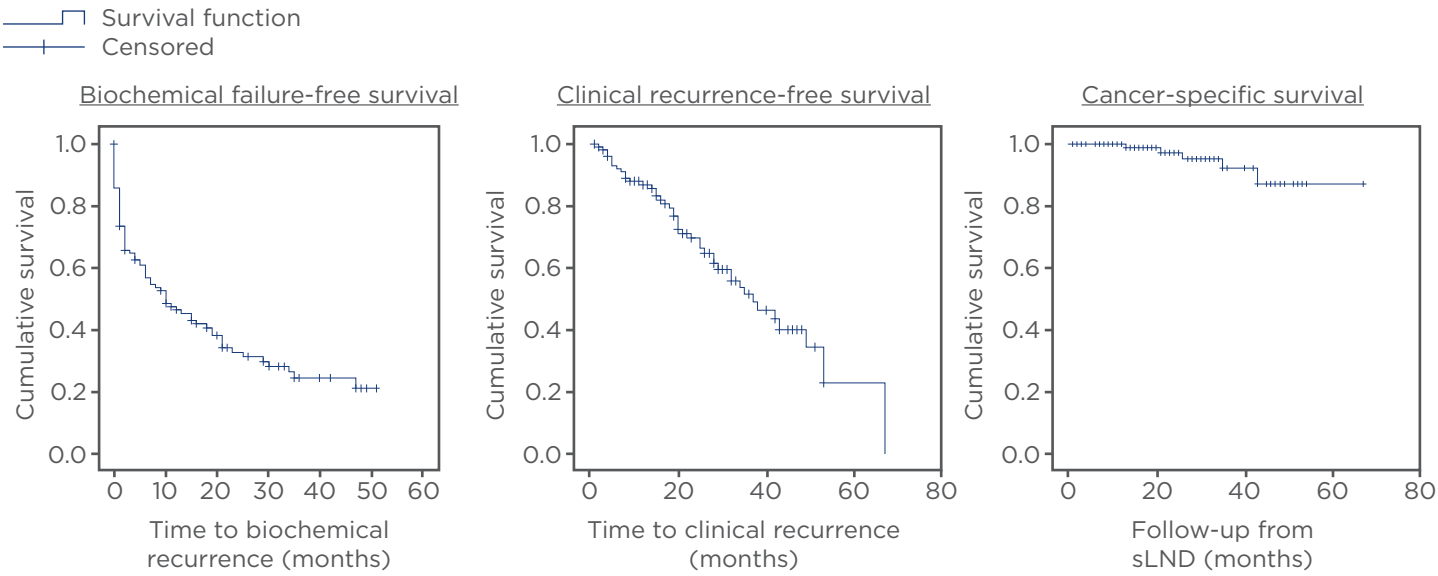


**Figure 1: Kaplan-Meier survival curves.**
sLND: salvage lymph node dissection.

## DISCUSSION

Recent advances in clinical imaging techniques, and in particular molecular imaging such as $^{11}$C-choline PET/CT, have allowed the identification of cases with oligometastatic nodal recurrence after radical therapy for PCa.[7,8] These are patients with presumed 'systemic' disease that is limited to the pelvic and/or retroperitoneal LNs. Traditionally, these these types of patients would have been

referred to HRT,[5] being at increased risk of death from PCa.[2] However, HRT is not a curative option and is burdened by non-negligible toxicity.[13] Moreover, many patients ultimately develop CRPC.[14]

In recent years, sLND has emerged as an appealing therapeutic alternative for this group of patients, showing favourable cancer-control outcomes in terms of delayed progression and postponement of HRT.[6,9-11,15-18] The rationale of this targeted surgery resides in the consideration that extended pelvic LN dissection at the time of radical prostatectomy is associated with more favourable survival rates in patients with low nodal disease burden, defined as up to two positive nodes.[6,11,19] In other words, nodal disease can still be considered regional in select cases. The question is whether limited nodal recurrence following local treatment with curative intent will be viewed in the same way. In other words, does sLND have curative potential, or at least therapeutic benefit as a cytoreductive measure?

Recent studies have analysed the outcomes of this controversial procedure. The first published large series of sLND was that of Rigatti et al.,[15] who analysed the data of 72 patients with BCR after RP associated with clinical nodal recurrence. Forty-one patients (57%) achieved a complete BR after sLND. In the entire cohort, the 5-year BFS, CRS, and CSS were 19%, 34%, and 75%, respectively.

**Table 2: Patient outcomes and complications.**

| Variable | All patients (N=106) |
|---|---|
| **Operative outcomes** | |
| Estimated blood loss (cc), mean±SD<br>Estimated blood loss (cc), median (range) | 395.3±434.3<br>250 (0–2700) |
| Length of hospital stay (days), mean±SD<br>Length of hospital stay (days), median (range) | 5.0±4.8<br>4.5 (0–31) |
| **Oncologic outcomes** | |
| Follow-up from primary treatment (months), mean±SD<br>Follow-up from primary treatment (months), median (range) | 95.9±50.6<br>86 (11–256) |
| Follow-up from sLND (months), mean±SD<br>Follow-up from sLND (months), median (range) | 25.7±15.1<br>22.5 (1–67) |
| Biochemical response immediately after sLND, n (%) | 54 (50.9) |
| Biochemical response at 22 months after sLND, n (%) | 34 (32.1) |
| Further treatments after sLND, alone or in combination, n (%)<br>• HRT (for at least a period of time)<br>• Chemotherapy<br>• RT on metastases | <br>71 (66.9)<br>13 (12.2)<br>12 (11.3) |
| Biochemical failure after sLND, n (%) | 72 (67.9) |
| Months to biochemical failure after sLND, mean (95% CI)* | 19.1 (15.0–23.1) |
| Clinical recurrence after sLND, n (%) | 43 (40.5) |
| Months to clinical recurrence after sLND, mean (95% CI)* | 38.1 (32.4–43.8) |
| Site of clinical recurrence, n (%)<br>• Local<br>• Nodal<br>• Bone<br>• Visceral | <br>4 (3.7)<br>24 (22.6)<br>16 (15.0)<br>2 (1.8) |
| Cancer-specific mortality, n (%) | 5 (4.7) |
| Months to cancer-specific mortality, mean (95% CI)* | 62.5 (58.7–66.3) |
| **Survival** | |
| Biochemical failure-free survival<br>• 2-years<br>• 4-years | <br>25%<br>22% |
| Clinical recurrence-free survival<br>• 2-years<br>• 4-years | <br>52%<br>25% |
| Cancer-specific survival<br>• 2-years<br>• 4-years | <br>92%<br>88% |

**Table 2 continued.**

| Variable | | All patients (N=106) |
|---|---|---|
| Complications | | |
| **Complication type** | **Clavien Grade** | **Overall, n (%)** |
| Lymphorrhoea | I | 11 (10.3) |
| Postoperative pain | I | 4 (3.7) |
| Pulmonary atelectasis | I | 1 (0.9) |
| Ileus | II | 1 (0.9) |
| Wound infection | II | 3 (2.7) |
| Pneumonia | II | 2 (1.8) |
| Pulmonary embolism | II | 1 (0.9) |
| Haemorrhage, with transfusions | II | 3 (2.7) |
| Lymphocele, requiring drainage | IIIa | 2 (1.8) |
| Haemorrhage, requiring embolisation | IIIa | 1 (0.9) |
| Wound dehiscence | IIIa | 1 (0.9) |
| Hydronephrosis, requiring ureteral stenting | IIIa | 2 (1.8) |
| Surgical reintervention | IIIb | 3 (2.7) |
| Rectovesical fistula | IIIb | 1 (0.9) |

*according to Kaplan–Meier survival estimates
RT: radiotherapy; HRT: hormone replacement therapy; sLND: salvage lymph node dissection; CI: confidence interval.

**Table 3: Cox regression models predicting CR after sLND.**

| Variable | Univariable | | Multivariable | |
|---|---|---|---|---|
| | HR (95%, CI) | p-value | HR (95%, CI) | p-value |
| **Time to BCR** | 1.00 (0.99–1.01) | 0.48 | - | - |
| **CRPC status** | 3.88 (1.04–14.49) | **0.04** | 32.4 (1.48–711.08) | **0.02** |
| **PSA at sLND (ng/mL)** | 1.26 (1.04–1.52) | **0.01** | 1.30 (1.04–1.62) | **0.01** |
| **Positive nodes, n** | 0.99 (0.93–1.07) | 0.97 | - | - |
| **Biochemical failure** | 6.48 (2.25–18.63) | **0.001** | 4.34 (1.26–14.96) | **0.02** |

BCR: biochemical recurrence; CRPC: castrate-resistant prostate cancer; PSA: prostate-specific antigen; sLND: salvage lymph node dissection; CI: confidence interval; HR: hazard ratio; CR: clinical regression.

In a recent update of this series, the same authors focussed on 59 patients with >5 years of follow-up. Complete BR was achieved in 35 patients (59.3%) after sLND. The estimated 8-year BFS, CRS, and CSS were 23%, 38%, and 81%, respectively.[9] Another report on sLND was published by Jilg et al.,[16] who evaluated the data of 52 patients with BCR after radical treatment associated with clinical nodal recurrence in PET/CT imaging. The authors excluded patients who received radiotherapy after primary treatment and before sLND. Adjuvant EBRT was administered following sLND in 27 cases (52%). Twenty-four patients (46%) had complete BR after surgery, and a 1-year BFS of 71.8%. In the entire cohort, the estimated 5-year CRS and CSS were 26% and 78%, respectively. Another series of sLND was recently published by Karnes et al.,[10] who analysed the data of 52 patients, 78.8% of whom had already undergone adjuvant or salvage therapy after RP. At a median follow-up of 20 months, 46.2% of patients had not received further treatments, 57.7% had PSA <0.2 ng/mL, and 34.6% were on HRT. Estimated 3-year BFS, CRS, and CSS were 45.5%, 46.9%, and 92.5%, respectively.

The outcomes of our multi-institutional report are in line with these results, with 2-year BFS, CRS, and CSS of 25%, 52%, and 92%, respectively. As with the aforementioned series, ~50% of our patients achieved complete BR after surgery, but the majority of our cohort developed BCR, with a mean time to BCR of 19 months. CRs were noted in a significant number of patients (40.5%), mainly at nodal or bone level. We do not know if these patients developed a true recurrence after sLND, or whether they already had more extensive systemic disease not detected by PET/CT. In this case, one might argue that these patients were not ideal candidates for surgery.

Several considerations, however, have to be made regarding the benefits of metastasis-directed surgery, at least for a sub-group of our patients. Among our cohort, 30% of patients remained with undetectable PSA after 2 years, and 60% were free from CR. Cancer-specific mortality was <5%. Another beneficial outcome of sLND is HRT-free survival: HRT can be avoided in these patients, or avoided for some time, sparing them from the side-effects of this treatment[13] and possibly delaying the time to CRPC.[14] Due to the retrospective design of our study, it was not possible to retrieve all data concerning the length and type of HRT administered to our patients. However, we observed that 71 of our patients (66.9%) received some form of HRT postoperatively, the duration of which was physician-driven but usually brief. The remaining 33.1% of cases remained HRT-free after sLND.[11]

When planning a sLND, the morbidity of this surgery must be taken into account. Most of the reported complications in the literature appear to be mild, the most frequent ones being lymphorrhoea (15.3%), fever (14.5%), and ileus (11.2%). The need for postoperative intervention due to severe complications was only sporadically reported.[6,10,15,16] Our data confirm that most postoperative complications are mild and self-limiting. However, around 10% of our patients experienced severe complications such as haemorrhage, hydronephrosis, or rectovesical fistulae, reminding us of the morbidity of this surgery. Again, accurate selection of patients is essential in order to ensure that the benefits of surgery outweigh the risks.

In an attempt to improve the selection of patients suitable for sLND, several authors have proposed factors that may help to identify the most-suited candidates.[6] Rigatti et al.[15] showed that pre-sLND PSA >4 ng/mL and the presence of retroperitoneal uptake at PET/CT represented independent preoperative predictors of CR, whereas the presence of pathologic nodes in the retroperitoneum, higher number of positive nodes, and complete BR after sLND represented postoperative independent predictors of CR.[15] Additionally, Jilg et al.[16] defined a Gleason score of 8–10 as an independent predictor of clinical progression. On the other hand, Karnes et al.[10] were not able to find any significant prognostic variable for systemic progression or CSS, likely as a result of the short follow-up or the heterogeneity of their cohort.

The results of our study are noteworthy, as CPRC status, PSA at time of sLND, and biochemical failure after sLND were all significant predictors of CR, both at univariable and multivariable analyses. According to our results, patients with CPRC status or high PSA values at the time of sLND may not be ideal candidates for metastasis-directed surgery. Surprisingly, the number of positive nodes at sLND was not associated with CR. The guidance of PET/CT is essential to select patients for sLND. The accuracy and limitations of $^{11}$C-choline PET/CT in this setting have been discussed elsewhere;[8] however, the diagnostic reliability of $^{11}$C-choline PET/CT seems to improve in the case of fast-PSA kinetics or increasing levels of PSA.[20]

Our study shares the limitations of previous reports, with its retrospective design and a median follow-up of 22.5 months. The heterogeneity of our cohort in terms of patient characteristics at sLND, surgical template, and postoperative management, limits the validity of our observations. The lack of a control group prevented us from reliably assessing the efficacy of surgery compared to traditional HRT. Hopefully, the results of an ongoing randomised, Phase II trial comparing eradication of oligo-recurrent disease versus active surveillance and androgen deprivation therapy at clinical progression[21] will help to shed light on this issue.

## CONCLUSIONS

According to the results of this multicentric cohort, which is the largest sLND series published to date, sLND represents a valid treatment option for selected patients with nodal recurrences. However, patients with CPRC status or high PSA values may not be the best candidates for a sLND. The morbidity of sLND and the significant risk of clinical progression despite surgery must be kept in mind during patient counselling.

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# GI CONNECT: AN INTERNATIONAL INITIATIVE FOR HEALTHCARE PRACTITIONERS IN GASTROINTESTINAL ONCOLOGY

GI CONNECT is a group of young, international, and independent experts in gastrointestinal (GI) oncology created in 2014, with a mission to identify and develop educational programmes for healthcare practitioners (HCPs) with an interest in cancers of the GI tract (www.giconnect.info/what-is-gi-connect). The group consists of 18 well-established medical oncologists from the USA, Europe, and Japan, who specialise in the treatment and research of GI malignancies (www.giconnect.info/meet-the-experts). The ultimate aim of GI CONNECT is to translate the latest scientific insights in the field of GI oncology into routine clinical practice, thus supporting HCPs worldwide to provide their patients with the best possible care according to international guidelines and cutting-edge developments in this area. The objectives of the group may include, but are not limited to: discussing the latest scientific and clinical insights in GI cancers, identifying educational needs for medical oncologists, developing global educational programmes utilising a number of contemporary platforms, and sharing the best clinical practices from international centres of excellence. GI CONNECT is an initiative of COR2ED, a European company in independent medical education which makes every possible effort to support and promote the work of the group.

Following its launch in 2014, GI CONNECT is now well established. Indeed, all of the group's activities are supported by a dedicated online platform (www.giconnect.info) and the group has been associated with the successful implementation of impactful educational programmes. These educational programmes include congress coverage and review and discussion of the most relevant recent publications, as well as sharing the most appropriate educational resources available. Although access to the group's online platform is free of charge, there are two sections, namely the public section and the members section. The public section contains presentations, a repository of educational programmes, and an introduction to the members of the group, while the members section is a restricted collaborative platform.

Over the years, GI CONNECT has completed a number of activities such as: coverage of three of the major oncology congresses, namely the Gastrointestinal Cancers Symposium (ASCO GI), the World Congress on Gastrointestinal Cancer (WCGIC), and the European Society of Medical Oncology-European Cancer Congress (ESMO-ECC); published newsletters; and developed three review manuscripts, which have been submitted for publication in peer-reviewed international oncology journals. It is of note that a summarising slide deck has been developed for each publication and they will be available and distributed with the link to the manuscript at the time of publication. For the current year, numerous activities are planned including congress coverage and the development of two new review manuscripts which will be submitted for publication. Furthermore, new types of exciting activities are in the pipeline for later in the year. This includes monthly video newsletters, search engine optimisation of the content available on the online platform to enhance the overall online visibility and maximise the number of visitors to the group's web portal, an active collaboration with the *European Medical Journal*, and engagement in social media in order to promote the group's activities, aiming to reach as many different individuals as possible around the world. Although the GI CONNECT group has only had an online presence since July 2015, it has become quite popular with HCPs who have an interest in the clinical management of GI cancers. As of February 2016, the website www.giconnect.info had already recorded 1,511 visits, 533 downloads, and 3,342 page views. It is also of interest that the newsletters associated with congress coverage appear to be a major hit. In particular, the newsletters produced for coverage of ESMO-ECC 2015 and ASCO GI 2016 were read by approximately 1,800 HCPs each.

The level of expertise and the volume of experience with the GI CONNECT group also provides an excellent opportunity for discussions on potential and future research activities, based on which the group develops proposals for research grants.

The future plans of the group are the continuous development of educational resources, the expansion of the membership of the group, as well as networking and developing collaborative partnerships with local, national, and international associations of (young) oncologists. The GI CONNECT experts do encourage HCPs worldwide, who are involved in the care of patients with GI malignancies, to feel free to network and get in touch with their questions. The long-term plan is to establish GI CONNECT as a standard reference for all HCPs involved in the clinical management of GI cancers.

The GI CONNECT initiative and its activities have been very well received by the international medical oncology and related professions community. It is of note that it has been estimated that more than 4,000 HCPs have benefitted from the resources provided as part of GI CONNECT. Based on the success of the current group, there has been enough encouragement to boost the desire for the creation and development of a similar group targeting HCPs in the field of genitourinary (GU) oncology. Given the experience gained from GI CONNECT during the last couple of years, an effective strategy for the creation and successful development of GU CONNECT is warranted. Nevertheless, it is expected with confident anticipation that GI CONNECT will continue to grow from strength to strength, achieving its major aims and objectives and thus having a positive impact on clinical practice in the area of GI oncology worldwide.

## Acknowledgements

All of the GI CONNECT activities are supported by an independent educational grant from Bayer Healthcare. Medical writing services were provided by Dr George Xinarianos of OncoMed Communications & Consultancy Ltd. (London, UK).

**Click below** to view the following:

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- **GI CONNECT: Interviews with GI CONNECT members**