EMJ HEMATOLOGY

US Edition — Vol 1.1 January 2020 emjreviews.com

+ ASH CONGRESS 2019

Reviewed

+ EDITOR'S PICK

Acute Chest Syndrome in Children with Sickle Cell Disease: Current Perspectives on Pathogenesis and Treatment

+ CONGRESS INTERVIEWS

Prof Theresa L. Coetzer and Dr Pierluigi Porcu speak about their current research and how ASH is supporting the ever-growing field of haematology

+ ARTICLE

Therapeutic Options in Myelodysplastic Syndromes Following Hypomethylating Agent Failure

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"EMJ Hematology US Edition is the newest addition to our haematology collection, and contains a review of ASH 2019, interviews with leading members of ASH committees, and a selection of impressive peer-reviewed articles"

Spencer Gore, CEO

+ ARTICLES

Editorial Board

VIEW IN FULL ←

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EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

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EMJ Hematol. US 1.1 2020

EMJ 4.4

A celebration of triumphant advancements throughout an outstanding year.

VIEW ALL JOURNALS ←

Welcome

A mere 6 weeks ago, the EMJ team were in to Orlando, Florida, USA, to attend the American Society of Hematology (ASH) Congress for the first time. With >30,000 delegates in attendance, this bustling congress was packed full of ground-breaking research and discussion of the most pressing topics in haematology. *EMJ Hematology US Edition* is the newest addition to our haematology collection, and contains a review of ASH 2019, interviews with leading members of ASH committees, and a selection of impressive peer-reviewed articles.

The stunning scientific programme matched that of its host city and spoilt the attendees for choice. We have hand-selected abstracts from the congress, and the authors themselves have provided a summary of the expert, inside knowledge of their studies for your reading.

Prof Theresa L. Coetzer and Dr Pierluigi Porcu both kindly answered our questions about their research, career paths, and roles as Chairs of the ASH International Members Committee and Foundation Committee, respectively. Prof Coetzer touched upon subjects such as the importance of a committee and the relationships with other organisations, as well as the future of haematology. In addition to his committee role, Dr Porcu gave us insight into the session that he presented at ASH 2019, and the importance of patient empowerment and how to achieve it.

The opportunity to learn about the latest findings in haematology does not just stop there, as also featured in this issue of *EMJ Hematology US Edition* is an array of excellent peer-reviewed articles. Topics discussed in the article include the mechanism, diagnosis, and management of pernicious anaemia; a case of Reed Sternberg-like cells in aggressive lymphoma; and acute chest syndrome in sickle cell disease.

We would like to thank all who contributed to this special edition, and who have subsequently assisted us on our biggest venture yet. We hope to see you at another great ASH event next year in San Diego, California, USA.

Spencer Gore

Chief Executive Officer, EMG-Health

Foreword

Dear Friends and Colleagues,

I am very happy to introduce to you the latest *EMJ Hematology US Edition*, an exciting addition to the EMJ catalogue in it being the first this year to provide, alongside its usual selection of peer-reviewed articles, a comprehensive review of a North American-affiliated congress: the American Society of Hematology (ASH) meeting in Orlando, Florida. ASH provides the clinical and research communities with one of their most valued platforms for disseminating the increasingly burgeoning advances in haematological research, making these annual meetings hotly anticipated throughout the year.

There were a number of ground-breaking advancements shared at the meeting. Results from the Phase III CANDOR trial indicate that a triple drug combination offers several more benefits over standard-of-care for the treatment of multiple myeloma, an important development in the clinical haematology field. Also presented for the first time was the engineering of an assay to monitor the lifetime of a blood clot, providing key insights as to how different drugs can treat different types of clot to bring about optimal clinical effects.

A varied selection of articles complements the ASH highlights. Belasen and Navada consider the therapeutic options available to patients with myelodysplastic syndromes following the failure of hypomethylating agents, addressing the approximately half of patients who do not adequately respond to these treatments. Manganas et al. contributed the case report of an exceedingly rare 'girdle syndrome' manifestation in a sickle cell patient. My Editor's Pick for this edition, however, is a comprehensive review by Uwaezuoke on the current perspectives for treatment of acute chest syndrome in children with sickle cell disease. The implications this comorbidity has for the patient in relation to their quality of life and the elevated mortality risk require increased attention for the haematology community, making this a highly relevant paper.

It brings me great joy and optimism to reflect on the advances being made in haematological research year-by-year, however much work is still needed to be done. I hope that the clinicians and researchers who read this edition of *EMJ Hematology* find something to take into their practice to further the fight against haematological disease.

Kind regards,

Doctor Emanuele Angelucci

Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale Policlinico San Martino, Genova, Italy

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Congress Review

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Congress Review

Review of the American Society of Hematology (ASH) Congress 2019

Location: Orange County Convention Center, Orlando, Florida, USA

Date: 7th – 10th December 2019

Citation: EMJ Hematol US. 2020;1[1]:12-25. Congress Review.

"The City Beautiful" proved to be an appropriate nickname for Orlando, Florida, USA, where the sun shined as >30,000 attendees flocked to attend this year's American Society of Hematology (ASH) Congress. Taking inspiration from Orlando's basketball team and the Wizarding World of Harry Potter™, ASH was a magical event that featured a scientific programme that held the attendees spellbound. The exhilarating atmosphere could be sensed as soon as you entered the convention centre, aptly reflecting that of a theme park.

The EMJ team, along with the attendees, were treated to an array of session types including satellite symposia, meet the scientist sessions, spotlight sessions, and trainee activities and services. In addition to these, >3,000 abstracts were presented, showcasing the latest developments in haematology. Award winning abstracts covered topics that ranged from RNA splicing defects in cancer-linked genes and tyrosine kinase inhibitor resistance in chronic myeloid leukaemia, to biomarkers in graft-versus-host-disease, highlighting the diverse nature of the ongoing excellent research.

This year, a new category of abstract was added to the collection: trials in progress. Covering trials that have not reached their primary endpoint yet, these abstracts facilitated the discussion and collaboration between investigators, translational researchers, statisticians, and regulators.

Our hand-picked selection of abstracts from ASH cover topics that include bone marrow neutrophils, the safety and efficacy of ferumoxytol, and the microenvironment of haematopoietic stem cells. The abstract summaries have been written by the authors' themselves, giving a first-hand account and novel insights into the research.

Three new clinical practice guidelines were announced at the meeting (sickle cell disease, venous thromboembolism, and immune thrombocytopenia), with two more to follow soon (von Willebrand disease and

acute myeloid leukaemia). Developed by leading clinical, methodological, and patient experts, these guidelines provide actionable recommendations on how best to treat these diseases.

Following on from previous years' attention on well-being, 2019 saw the first appearance of the ASH Wellness Studio. Throughout the meeting, attendees could visit the Wellness Studio to watch short (10–20 minutes) sessions on topics that contribute to the development of healthy habits. Among the topics delivered were actions that could be taken to improve sleep quality, avoid mental fatigue and burnout, and reduce stress.

Another initiative making its first appearance was the powernap pods. Attendees were encouraged to take a short 20-minute power nap in the specially designed chairs that lie you in a zero-gravity-like position. The pods popularity was not just the result of a free powernap blanket, but the fact that a powernap helped overcome jetlag for those who had travelled from around the world to attend ASH, and thus reduce tiredness and allow full participation in all that was available at the congress.

ASH put on a fantastic event this year, spoiling us for choice at every opportunity and making the most of the 2 million square feet that was the Orange County Convention Center. The rapid growth of the congress reflected the city that played host, but what will next year in San Diego, California, USA, hold? We are sure that it will be another unmissable ASH event. Till then, we present our review of ASH 2019.

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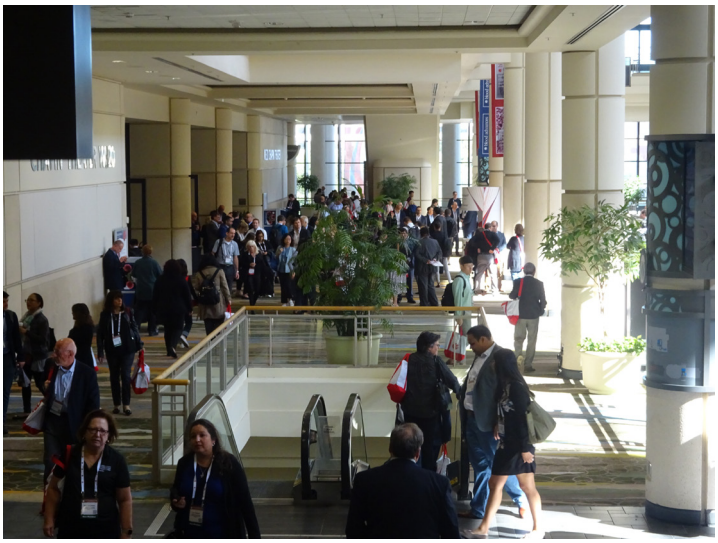

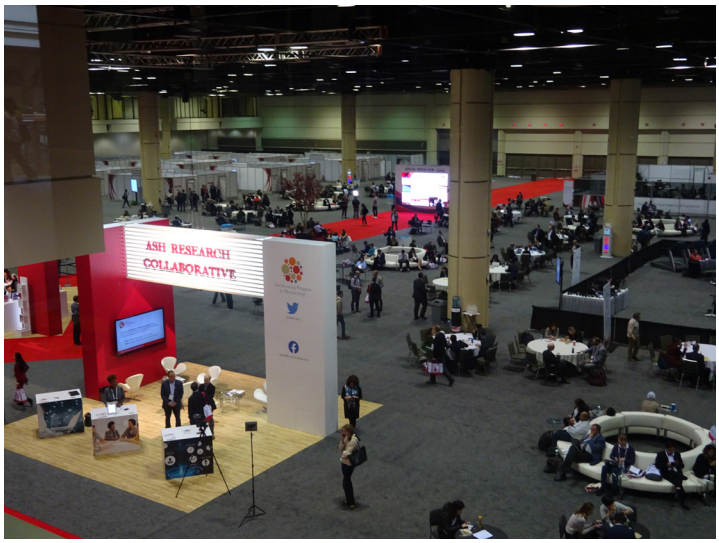

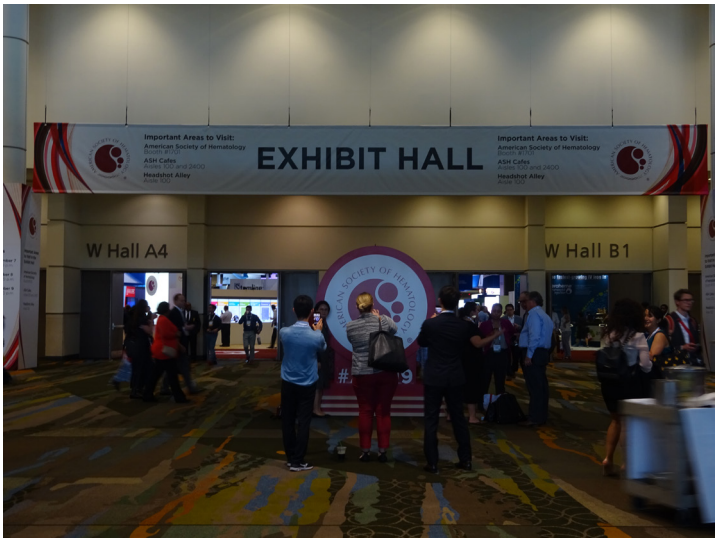

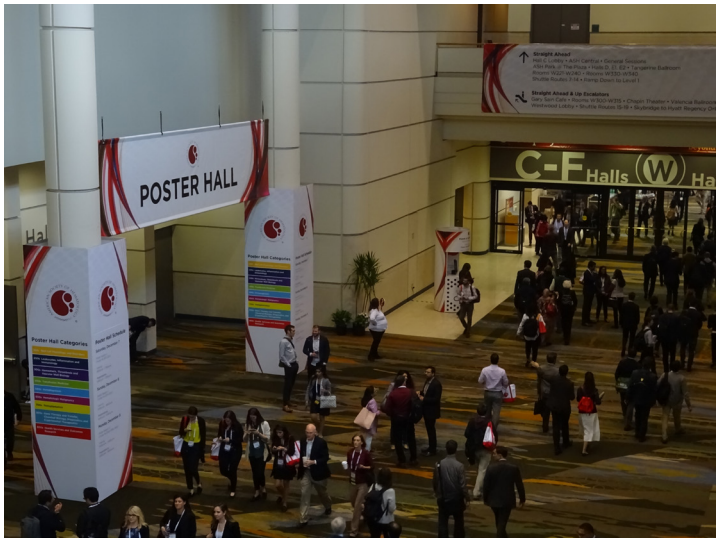

ASH 2019 REVIEWED →

New Gene Therapy Approach for Sickle Cell Disease Successfully Targets Fetal Haemaglobin Retention

"We are seeing a remarkable reproducibility of the treatment effect in all of the patients treated to date..."

IN LATER stages of pregnancy and in the initial weeks following birth, infants produce so-called fetal haemoglobin, a derivative that is especially suited for the lower oxygen environment present in the womb. Importantly, fetal haemoglobin inhibits the production of sickled red blood cells, making this molecule an attractive target to be considered when designing gene therapy approaches for the treatment of this serious condition. Now, researchers from Boston Children's Hospital, Boston, Massachusetts, USA, have revealed encouraging results from a trial in which three adult patients received a transfusion of genetically engineered stem cells capable of reactivating fetal haemoglobin production. The results were presented in a press release at ASH dated 10th December, 2019.

In the study, the three patients were treated with a single injection of a drug that localised haematopoietic stem cells to the blood vessels where they were extracted. The captured cells were transduced with an inactivated virus designed to reduce expression of *BCL11A*, whose protein product has been shown to represent a key regulatory factor in the switch from fetal to adult forms of haemoglobin. Following a freezing step in which the cells underwent rigorous safety testing, the patients received controlled doses of chemotherapy to eliminate the resident haematopoietic stem cells in the bone marrow, preceding intravenous infusion of the modified stem cells.

Following an engraftment period and post-treatment follow-up, all three patients (who are 18, 10, and 9-months post-infusion) now harbour normal or near-normal haemoglobin levels and are producing fetal haemoglobin in quantities sufficient to prevent sickling. Additionally, no therapy-related adverse effects, outside of those commonly associated with autologous haematopoietic stem cell transplantation, have been reported. "We are seeing a remarkable reproducibility of the treatment effect in all of the patients treated to date," commented Lead Researcher Dr David A. Williams. "We are very encouraged by the results we are seeing with this scientifically innovative approach to gene therapy for sickle cell disease."

Maintenance of First Remission Leads to Improved Overall Survival in Acute Myeloid Leukaemia Patients, Results Suggest

VALIDATION of maintenance therapies for the extension of initial treatment response in patients with acute myeloid leukaemia (AML) has to date been ambiguous. Minimising the risk of relapse is clearly the primary goal, however definitive influences on survival benefit have not been robustly demonstrated. Now, results from a Phase III study presented in an ASH press release dated 10th December 2019 have shown an investigational oral form of azacytidine therapy to significantly improve overall survival in newly diagnosed, remissive AML patients following standard induction chemotherapy.

Approaches to post-remission management in AML patients is limited to either observation or stem cell transplant, the latter of which is not currently a scalable option across the patient demographic. This period represents a window in which further intervention can maximise the benefits of prior treatment and lead to long-lasting patient remission.

In the QUAZAR trial, 472 patients with AML aged 55–86 years and with either intermediate or poor-risk cytogenetics were enrolled from centres across 23 countries. Upon achieving complete response within 4 months of induction chemotherapy, patients were randomised to receive either 300 mg of CC-486 (i.e., oral azacytidine therapy) or placebo once daily for 14 days of a 28-day cycle including supportive care until disease relapse. Overall survival, relapse-free survival, safety and tolerability, and health-related quality of life were tracked.

Following a median follow-up of 41.2 months, a 31% decrease in risk of death among the CC-486-treated patients was reported compared to placebo, with median overall survival also showing average improvements of 7.9 months. CC-486 also conferred a significantly lower risk of relapse compared to placebo (10.2 months versus 4.8 months, respectively), and strikingly, these benefits were consistent across varying subgroups of patients, including those defined by the number of chemotherapy cycles received.

Dr Andrew H. Wei, Lead Study Author from the Alfred Hospital, Melbourne, Australia, highlighted that "the QUAZAR shows that, rather than observing patients and waiting for them to relapse, we can now actively engage in trying to reduce relapse risk and improve survival in the post-remission phase."

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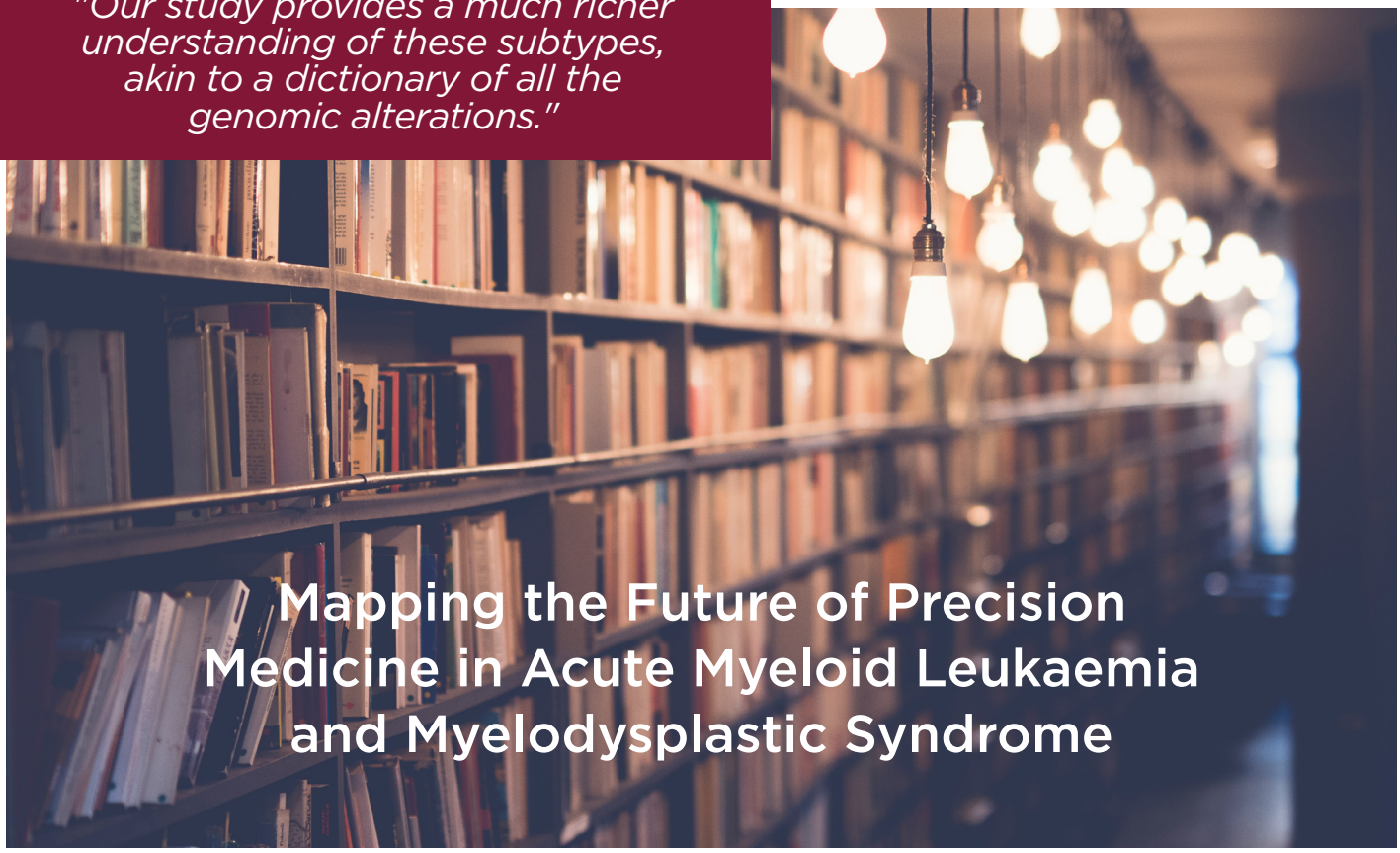
Sutimlimab Offers Favourable Effects on Symptoms of Cold Agglutinin Disease

COLD agglutinin disease (CAD) is an autoimmune disease characterised by the onset of severe anaemia, fatigue, increased risk of blood clotting, and early death caused by the body's immune system mistakenly attacking and destroying its own red blood cells. Diagnosis of this rare condition occurs after 60 or 70 years of age and currently has no approved treatment options. A new study, described in a ASH press release dated 10th December 2019, investigated the effect of sutimlimab on symptoms and pathology of CAD.

The classical complement pathway, which is typically overactive in CAD patients, can be blocked with the monoclonal antibody sutimlimab. The authors from University of Duisburg-Essen, Duisburg, Germany, explored the actions of sutimlimab designed to prevent C1s proteins from activating and whether this would be effective in retaining erythrocytes and ceasing haemolysis. The study recruited 24 patients with an average age of 71 years, all of whom had active CAD with haemoglobin levels ≤10 g/dL, above the normal range for total bilirubin, and a prior 6-month history of at least one blood transfusion. The primary endpoint of the study was an increase of two or more in haemoglobin or total haemoglobin level of 12 or higher, and no use of blood transfusion between Weeks 5 and 26. Intravenous administration of sutimlimab took place over 26 weeks and disease symptoms were assessed at Weeks 23, 25, and 26.

After the initial sutimlimab dose, haemoglobin levels rapidly increased and by the first week, quality of life scores improved. By the third week, most of the study group exhibited haemoglobin levels >11 g/dL and normalised total bilirubin. A serious adverse event was experienced by 29% of patients and at least one adverse event was experienced by 92% of all patients, many events of which were unrelated to sutimlimab. Adverse events related to the drug including swelling, higher blood pressure, and rhinorrhoea were deemed nonserious. A commonly encountered limitation for studies of rare diseases such as this one is sample size; furthermore, the absence of a placebo group was acknowledged by the authors to have limited the study. The investigation showed that response to sutimlimab administration over 26 weeks in CAD patients resulted in improved symptoms for CAD including improved haemoglobin levels, less fatigue, and fewer required blood transfusions.

"Our study provides a much richer understanding of these subtypes, akin to a dictionary of all the genomic alterations."



Mapping the Future of Precision Medicine in Acute Myeloid Leukaemia and Myelodysplastic Syndrome

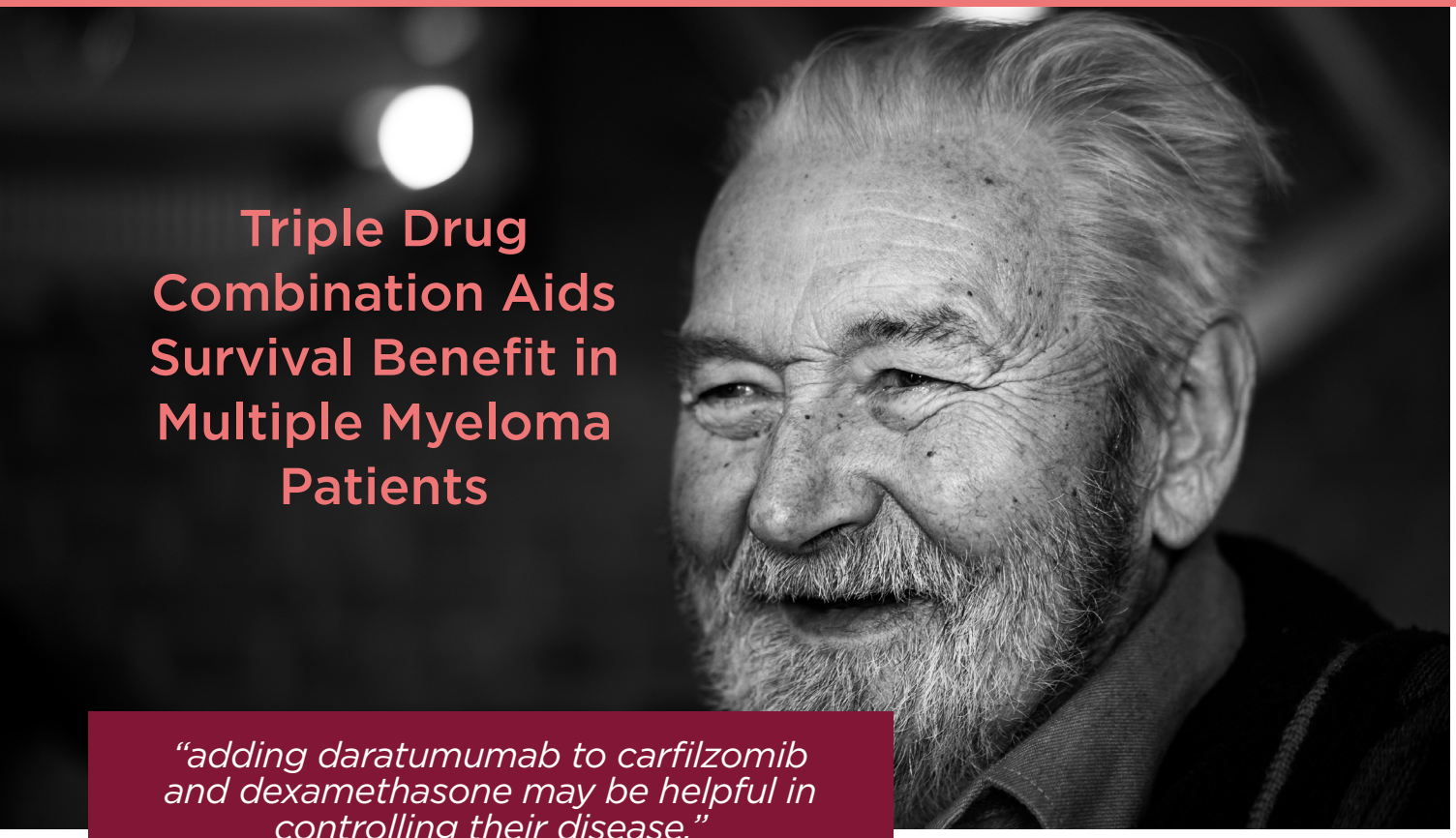
MORE than 1,300 patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) have been included in a comprehensive analysis of genomic sequencing and gene expression profiles, the first study to take a genome-wide approach that is unbiased in order to discover the links between genes, gene expression, the physical form of cancer cells, and patients outcomes. These findings were presented in a press release at ASH dated 10th December, 2019.

Blood and bone marrow samples were taken from 598 AML patients and 706 MDS patients. Researchers sequenced the DNA and RNA from these samples before combining the results with data on the health outcomes and physical features of each individual's cancer. The findings from this were multifaceted: not only was the team able to corroborate known genetic subtypes of the cancers studied that are driven by chromosomal lesions or mutations, but they also discovered some brand new associations. Pairs of genetic alterations appeared to impact prognosis if they occurred simultaneously in patients, notably combinatorial mutations in *NPM1* and cohesion genes which are ordinarily associated with good prognosis.

An additional discovery by the team was the similarity in the genes of certain cancers that are morphologically very distinct from one another. This suggests that standard approaches to cancer diagnosis and treatment could be combined with genetic analysis to offer a much more detailed picture of the cancer than morphology alone.

In an ASH press release dated 10th December 2019, Dr Ilaria Iacobucci, St. Jude Children's Research Hospital, Memphis, Tennessee, USA, commented of the study: "Our study provides a much richer understanding of these subtypes, akin to a dictionary of all the genomic alterations. It also underscores the value of having comprehensive genomic information at the start of treatment to remove uncertainty and help clinicians better understand a patient's outlook." The findings of the study having meant that a complete genetic reference for AML and MSD subtypes is now available to guide future research into the development and treatment options of these diseases. Precision medicine for these and other diseases could be the next avenue of research, as the study has demonstrated that full genome-sequencing is feasible and helpful for identifying disease subtypes and prognosis.

Triple Drug Combination Aids Survival Benefit in Multiple Myeloma Patients



"adding daratumumab to carfilzomib and dexamethasone may be helpful in controlling their disease."

MULTIPLE myeloma, a rare cancer that affects the bone marrow and alters the blood's plasma cells, currently has no cure but treatment can control the disease for several years. According to an ASH press release, a recent study found that adding daratumumab to carfilzomib and dexamethasone to treat patients with relapsed or refractory multiple myeloma resulted in increased survival. These results were presented in a press release at ASH dated 10th December, 2019.

Present treatment has greatly improved the life expectancy of patients diagnosed with multiple myeloma, yet a great proportion of the patients eventually relapse. A common treatment option is lenalidomide and bortezomib; although this improves survival outcomes, researchers state that many patients prescribed these agents experience disease progression or are forced to stop due to toxicity. Lead author of the study Dr Saad Z. Usmani, Levine Cancer Institute, Charlotte, North Carolina, USA, stated: "The majority of patients have disease progression on lenalidomide and, of the six treatment combinations that are currently approved in this setting, four have lenalidomide as part of their treatment combination." He further stated that there is a need for novel treatment options for these patients as it makes little sense to re-challenge a patient with something they are progressing on just by adding another drug.

In their trial, the researchers investigated whether adding daratumumab to the current standard of care (carfilzomib plus dexamethasone) would improve progression-free survival, the time from randomisation to disease progression, or death from any cause. A total of 466 patients with relapsed or refractory multiple myeloma were recruited. These patients previously received therapy and were enrolled in the open label, Phase III trial CANDOR. Patients were randomised 2:1 to receive carfilzomib, dexamethasone, and daratumumab, or carfilzomib and dexamethasone, and of these patients one out of three were lenalidomide refractory.

Overall response rate, minimal residual disease, overall survival, and safety were evaluated, and results showed that the addition of daratumumab resulted in a greater response compared to carfilzomib and dexamethasone alone. Adverse events included thrombocytopenia and cardiac events, with heart failure being lower in the three-drug treatment group. Dr Usami concluded that providing lenalidomide free treatment for patients who do not respond to it is a real clinical need and that: "adding daratumumab to carfilzomib and dexamethasone may be helpful in controlling their disease." Furthermore, because myeloma is a heterogenous disease, optimal disease control requires targeting different mechanisms of action to control the disease more effectively.

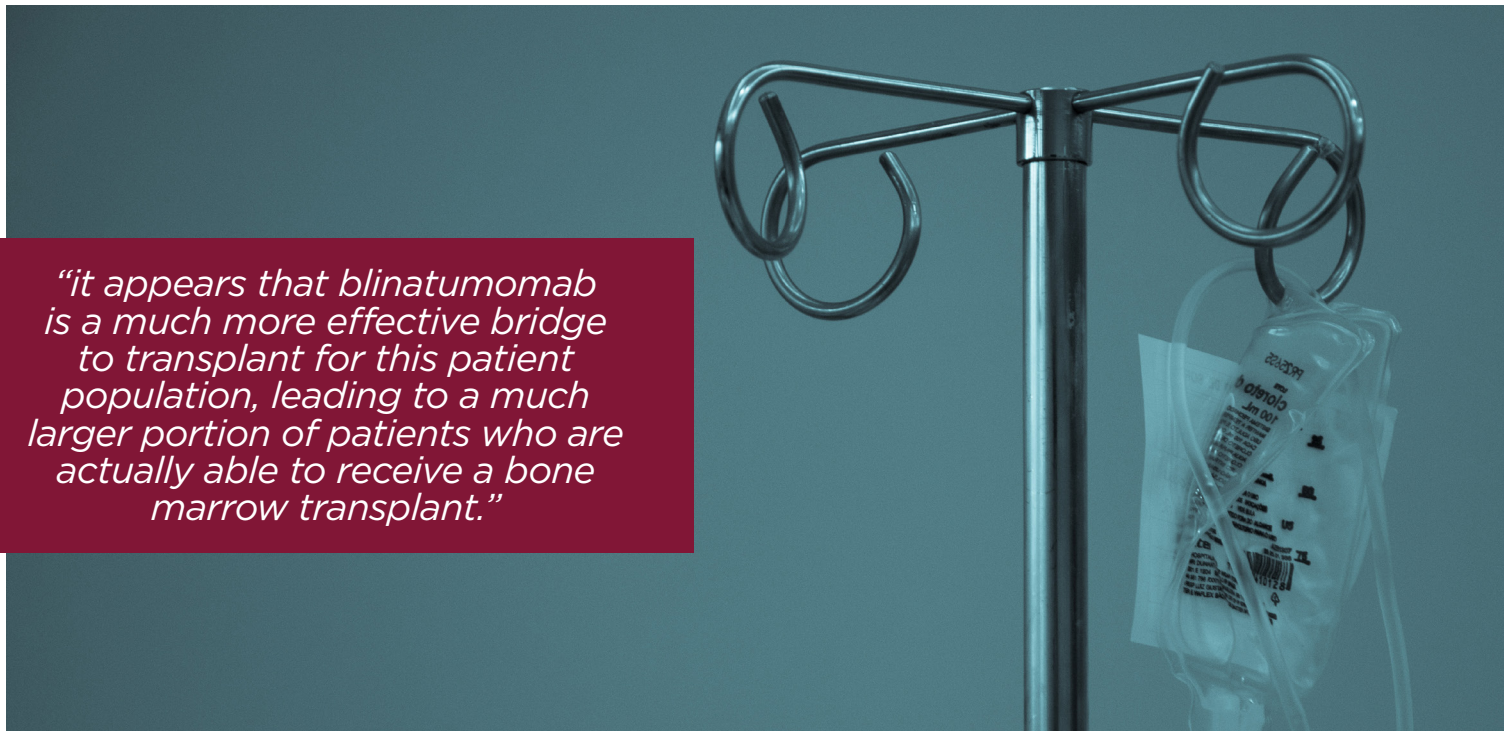
Immunotherapy versus Chemotherapy for B-Acute Lymphoblastic Leukaemia

RELAPSED B-acute lymphoblastic leukaemia (B-ALL) in paediatric patients preparing to undergo bone marrow transplant was more effectively treated with the immunotherapy drug blinatumomab, as opposed to standard chemotherapy, according to the results of a recent study presented at 61st ASH Annual Meeting in Orlando, Florida, USA and reported in a press release dated 10th December 2019.

The study, led by Dr Patrick Brown of the Johns Hopkins Kimmel Cancer Center in Baltimore, Maryland, USA, assessed patients receiving 3 months of chemotherapy prior to bone marrow transplant. Dr Brown and his team identified 208 patients with high or intermediate risk B-ALL and divided them into a blinatumomab treatment group and standard chemotherapy group. A median follow-up of 1.4 years revealed the advantages of blinatumomab treatment; patients showed 59% disease-free survival compared to 41%, overall survival was 79%, and 73% were able to proceed to transplant, compared with 41%, 59%, and 45% for chemotherapy, respectively. The interim analysis of the trial halted enrolment as the success of blinatumomab were significant enough to recommend it as the new standard of care.

Blinatumomab, a bispecific T-cell engager antibody construct, exhibits its action by causing the body's immune cells to find and destroy cancerous cells. In contrast, chemotherapy works to destroy all growing cells in the body; this causes toxic side effects such as sepsis and fevers, as noncancerous cells are also targeted. Though blinatumomab is approved by the U.S. Food and Drug Administration (FDA), this study was the first to give evidence of its ability to benefit paediatric patients with minimal residual disease who are awaiting transplant. Dr Brown recalled his past experiences with this disease, stating "it appears that blinatumomab is a much more effective bridge to transplant for this patient population, leading to a much larger portion of patients who are actually able to receive a bone marrow transplant."

The next steps to be taken by Dr Brown and his team will be to monitor the long-term effects of blinatumomab, alongside studies to investigate the potential of optimising the immunotherapy earlier in the B-ALL treatment cycle.



"it appears that blinatumomab is a much more effective bridge to transplant for this patient population, leading to a much larger portion of patients who are actually able to receive a bone marrow transplant."

New 'Choosing Wisely' List Developed to Reduce Unnecessary Testing in Paediatric Patients

TO CAMPAIGN against avoidable tests and procedures in the paediatric population, ASH and the American Society of Pediatric Hematology/Oncology (ASPHO) launched a list of recommendations as part of their 'Choosing Wisely' campaign. This was presented at this year's ASH Annual Meeting in Orlando, Florida, USA, as part of a press release dated 9th December 2019.

A panel was assigned the task of creating the list, made up of two co-chairs representing ASH and ASPHO, five members chosen by each organisation, and one delegate acting as the Choosing Wisely advisor on methodology. The guiding principles used to make their recommendations was avoiding harm to patients, creating evidence-based recommendations, an evaluation of the costs and frequency of tests and treatments, producing recommendations in the clinical scope of the haematologist, and a contemplation of the potential impact of the recommendations.

The panel agreed upon five evidence-based recommendations for haematologists and their patients. The first recommendation is not to perform preoperative haemostatic testing in a healthy child with no personal or family history of bleeding and the second is not to transfuse platelets in a nonbleeding paediatric patient with a platelet count >10,000 per mcL, the exception being when signs or symptoms of bleeding are present or if invasive procedure is necessary. The third recommendation states that children with venous access associated thrombosis, without a positive family history, should not be tested for thrombophilia. The fourth recommends not to transfuse packed red blood cells as treatment for asymptomatic paediatric patients with iron deficiency anaemia if there is no signs of active bleeding or haemodynamic instability. The fifth and final recommendation relates to granulocyte colony stimulating factor, in that it should not be administered routinely in paediatric patients with asymptomatic autoimmune neutropenia in the absence of recurring or acute bacterial and/or fungal infections.

> *"it is very exciting to see a list focussed on the unique needs of our patient population."*

It is the hope of ASH co-chair Dr Sarah O'Brien, of the Nationwide Children's Hospital, Columbus, Ohio, USA, that haematology practitioners will learn these recommendations and teach them to their colleagues. She says, "it is very exciting to see a list focussed on the unique needs of our patient population."

A Bright Future for Sickle Cell Disease Patients

PRESENTATIONS at this year's ASH meeting in Orlando, Florida, USA, demonstrated the huge efforts being made by researchers to understand sickle cell disease (SCD) and how best to treat patients. Three studies were presented, reporting great strides forward made this year in understanding the underlying mechanisms that cause the disease, how to reduce pain for children during vaso-occlusive crises, and fragmentation of care between childhood and adulthood. ASH is passionately committed to supporting research in this area and the announcement of these study results comes after a number of years of work across the USA and internationally supporting this important cause with the publication of clinical guidelines for SCD management and care and the development of the Sickle Cell Disease Coalition.

The first of the studies announced was an exploration into the role of protein nuclear factor I X (NFIX) in the development of SCD. The team found that NFIX prevents adult erythroblasts from producing fetal haemoglobin, and therefore from producing healthy red blood cells. To test this, the team suppressed NFIX in adult erythroblasts and found that this allowed the cells to produce fetal haemoglobin. Although it's not yet clear whether NFIX directly or indirectly represses production of fetal haemoglobin, future studies will endeavour to learn more about this process.

For children experiencing vaso-occlusive crises, pain is often present and this was the subject of the second study reported in the press release. The study, which was the first of its kind conducted in Nigeria to test the use of oral arginine supplements for acute pain control, enrolled 68 children who had been hospitalised for vaso-occlusive crises in two hospitals in Abuja, Nigeria, and administered oral arginine to half of the participants while half received placebo. Doses were given every 8 hours until the patient was discharged from hospital or up to 15 doses. The group who received arginine showed a faster decline in pain as well as requiring less pain medication overall (the decrease in total opioid use was not statistically significant), and their crises ended sooner than for those in the placebo group. Half of the participants who received arginine supplements were discharged by Day 5 on their hospital admission, compared to only a quarter of the participants who received the placebo. These results indicate that oral arginine supplements should be tested further to see whether administration at the onset of a crisis could prevent hospitalisations and readmissions.

"These studies together illustrate how progress is being made all the way from the laboratory bench to dissemination and implementation of better treatments and systems-level quality improvements."

In the final study, researchers looked at the fragmentation of care for patients with SCD during childhood and adulthood, noting that children with the disease are much more likely than young adults to receive consistent care. In the study, it was found that 60% of children with the disease were receiving care from just one facility, whereas 78% of young adults with the disease had been admitted to multiple care facilities. The study analysed the health records of almost 7,000 people who had been diagnosed with SCD and attended medical centres in California, USA, between 1991 and 2016. The participants were grouped into three separate age ranges: 10–17 years, 18–25 years, and 26–33 years. As patients moved from the first age group to the second, fragmentation of care became more frequent, however age was not the only indication of this: patients without health insurance, patients who were hospitalised more frequently, and who did not attend specialist SCD centres were also more likely to receive fragmented care. Fragmentation of care, however, was not independently associated with an increased risk of death.

Commenting on the studies, Dr Julie Panepinto, Medical College of Wisconsin, Milwaukee, Wisconsin, USA, said: "These studies together illustrate how progress is being made all the way from the laboratory bench to dissemination and implementation of better treatments and systems-level quality improvements."

Socioeconomic Factors at Play in Cancer Survival Rates

CHILDREN with acute myeloid leukaemia who were from low-income neighbourhoods were found to be 2.4 times more likely to die following treatment, according to a study presented at ASH 2019 and discussed in a press release dated 7th December 2019. The study, which analysed data from around 1,500 patients who had participated in clinical trials, compared low, middle, and high-income neighbourhood children and found this disparity between socioeconomic status and survival rates.

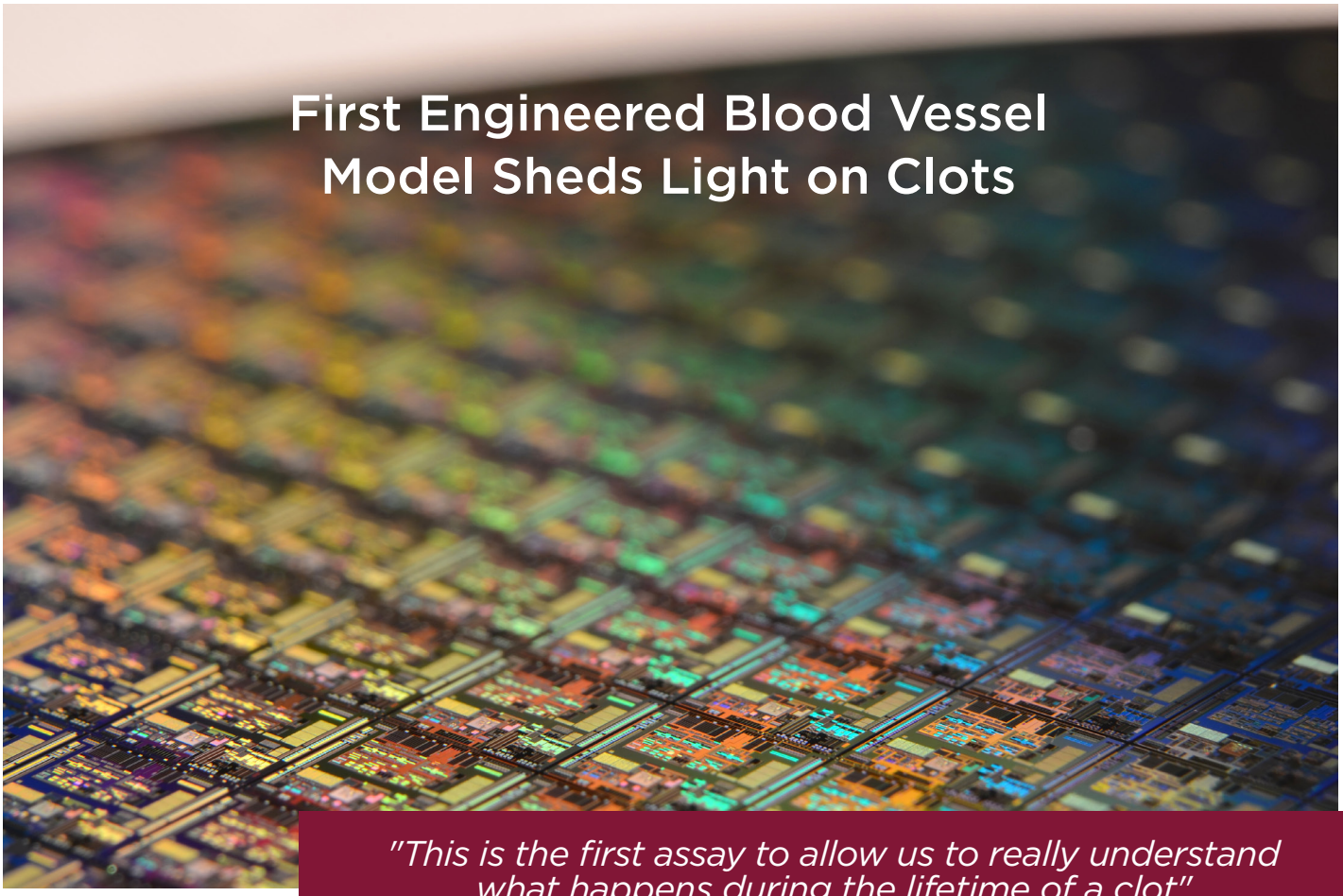
Clinical trials, by nature, should provide treatment that is consistent for all patients within the group; therefore, these findings were particularly concerning for the researchers. This could point to factors that arose which were not related to the chemotherapy. "We expected there to be a difference, but the degree of difference is quite substantial," explained Dr Lena E. Winestone, UCSF Benioff Children's Hospital in San Francisco, San Francisco, California, USA, who was the lead author.

Within the analysis, data was gathered pertaining the patients' neighbourhoods, including median income and education status. Results showed that socioeconomic factors could be used as significant predictors of death. Race, known-biologic factors, and insurance status were also accounted for. Survival time of 5 years post-diagnosis was 68% for middle and high-income patients, 61% in low-income patients, and 43% in patients who were living in poverty.

While the researches did not identify the reasons for a higher death rate, one suggestion is toxic stress: a known association of poor socioeconomic status. This could play a role in chemotherapy response or immune recovery. The team stated plans to examine cause of death in the patients to determine whether the higher risk was treatment or disease related.

Dr Winestone called for collection of socioeconomic status in clinical trials going forward, to provide more accurate information and avoid reliance on neighbourhood data: "If we could gather that information, it would allow us to dig deeper into the question of how someone's circumstances outside of the clinical aspects of their disease impact their health outcomes."

First Engineered Blood Vessel Model Sheds Light on Clots



"This is the first assay to allow us to really understand what happens during the lifetime of a clot"

BLOOD clots commonly lead to heart attacks, stroke, pulmonary embolism, and a host of other life-threatening events. Clots have been hard to investigate for sustained periods of time, until now. Researchers from the Emory University School of Medicine, Atlanta, Georgia, USA, have engineered microvasculature on a chip that facilitatestheinvestigationofthelifetimeofaclot,the data of which was presented in an abstract session at ASH 2019 and discussed in a press release dated 7th December 2019.

Typically, blood clots are treated with drugs that cause bleeding, restoring blood flow and avoiding ischaemic injury; however, this is a fine balancing act because there is a risk of excessive bleeding. Understanding how a clot develops, progresses, and resolves will provide key insights into how a drug should function to bring about the best clinical effect. Lead study author Dr Yongzhi Qiu commented: "This is the first assay to allow us to really understand what happens during the lifetime of a clot – looking at it from the time it forms, to see how clots get better, either on their own or when adding different clot-busting drugs."

In a similar manner to culturing skin cells on a two-dimensional slide, microvasculature on a chip was engineered by growing endothelial or blood vessel cells in a tissue-like material to form three-dimensional micro-vessels with branches. The technology is capable of modelling many aspects of a natural blood vessels biophysical environment including vessel size, geometry, wall stress, and the stiffness of the surrounding tissue.

The researchers have already discovered new information about clot formation and dissolution. Data indicate that inflammation has a bigger involvement than first thought, including the induction of protein deposits that initiate clot formation and altering clot-dissolution time.

"What we are learning is that not all clots are created equal, and not all clots should receive the same type of therapy – some therapies are going to be better for certain clots than others," concluded Dr Qiu. This assay has the potential to help develop better and targeted antithrombotic and anticoagulant therapies, especially for thrombosis in inflammatory diseases such as lupus, rheumatoid arthritis, and inflammatory bowel disease.

Congress Interviews

We spoke to Prof Theresa L. Coetzer, the Chair of the ASH International Members Committee, and Dr Pierluigi Porcu, the Chair of ASH Foundation Committee to find out about their current research and how ASH is supporting the ever-growing field of haematology.

Prof Theresa L. Coetzer

Chair of the International Members Committee at the American Society of Hematology (ASH)
Research Professor at the University of the Witwatersrand, Johannesburg, South Africa

Q1 You have a Bachelor of Science and Doctor of Philosophy in biochemistry. At what point did you know this was a particular passion of yours?

At school I was very interested in biology and science, and at university I realised that the field of biochemistry holds immense promise and will be at the forefront of exciting new discoveries.

Q2 A specific interest of yours is malaria and inherited haemolytic anaemias. What is it about this field of research that fascinates you?

I think the red blood cell is the most amazing cell in the body. When I started my PhD there was very little known about the role of the red cell membrane in the pathogenesis of inherited haemolytic anaemias, so there was lots of scope for new discoveries. The field developed rapidly, and novel molecular biology tools enabled cloning of the genes encoding the membrane proteins. By studying patients, it became possible to identify pathogenic mutations.

I have always been interested in malaria, especially since I live in a malaria endemic country and the main burden of malaria is in sub-Saharan Africa. Evolutionary pressure from the infection has modified the human genome and resulted in numerous inherited disorders of red blood cells, which provides a natural link between the two fields. The malaria parasite infects the red blood cell and interacts with the membrane, so it became a logical extension of my research. My fascination with this incredibly successful pathogen has increased over the years, and I expanded my studies to investigate the biology of the parasite with an overall aim of identifying new drug targets using cutting edge 'omics' technologies,

including gene editing. I am currently involved in a South African malaria drug discovery consortium focussing on identifying compounds that block transmission of the parasite to the mosquito vector, which will assist global efforts in eliminating this deadly disease.

"I realised that the field of biochemistry holds immense promise and will be at the forefront of exciting new discoveries."

Q3 This has been the 61st ASH Annual Meeting. Why do you believe this has become such a successful event that is eagerly anticipated year on year?

The ASH Annual Meeting showcases the latest discoveries in haematology and represents the premier haematology meeting in the world since it only accepts high-quality abstracts that reflect novel unpublished data advancing the field. In addition, the education programme provides a superb update on the latest aspects of the chosen topics, and special sessions are held for trainee haematologists. The meeting has global impact and attracts the top echelon of scientists and clinicians that inspire all attendees, including young emerging researchers and trainees.

Q4 Which session do you consider to have been the most pivotal this year at the ASH Annual Meeting?

There were numerous excellent sessions at ASH this year that highlighted important discoveries and it is difficult to single out one particular session. However, the Presidential Symposium on big data and precision medicine, as well as the ASH-EHA Joint Symposium on haemato-metabolism provided important indications of where the field is going and how different disciplines are being integrated into haematological research endeavours.

Q5 One role of the International Members Committee is to maintain ASH communication with Health Volunteers Overseas (HVO). Could you tell us about why this is such a crucial relationship?

HVO is an organisation with a longstanding record of excellence in sending volunteers to low and middle-income countries to train local personnel. It is important for ASH to partner with HVO since HVO manages the logistics and administrative aspects of the volunteer assignments. The International Members Committee has representation on the HVO-ASH steering committee and can thus provide input and guidance on the haematology programme.

Q6 As the Chair of the International Members Committee, how do you perceive haematology treatment to differ across the globe?

Diagnosis and treatment of patients with haematological disorders differ markedly across the world and reflect the inequality of society. The high cost of new therapies precludes their use in resource-constrained countries, which unfortunately means that patients in these countries do not benefit from research advances and die because they cannot afford treatment.

Q7 A main focus of the International Members Committee is the educational programmes you are involved in. Are there any exciting programmes or opportunities ongoing currently?

The International Members Committee oversees several unique programmes that focus on training and education. For low and middle-income countries the well-established Visitor Training Program (VTP) and the related Latin American Training Program (LATP) build haematology capacity by providing funding for haematology-related healthcare professionals to receive up to 12 weeks of training on a specific topic or technique, anywhere in the world (VTP) or at specific centres in Latin America (LATP). Training occurs under guidance of an ASH mentor and awardees implement their new knowledge

upon return to their home country. The HVO-ASH programme complements this by sending volunteer haematologists to train local individuals on-site in specific locations in low and middle-income countries. These programmes endeavour to mitigate the lack of expensive diagnostics and therapies by teaching local doctors to make the best use of available drugs and basic diagnostic techniques.

The International Outreach Initiative provides hospitals and universities in resource-constrained countries with free online access to educational materials, which help to address their haematological needs.

In addition, ASH has recently launched very exciting new international initiatives to expand their global portfolio. These include the Global Capacity-building Showcase, which highlights collaborative efforts to educate and train individuals in low-to-middle income countries, and the Global Research Award, which supports future leaders from countries at all levels of development.

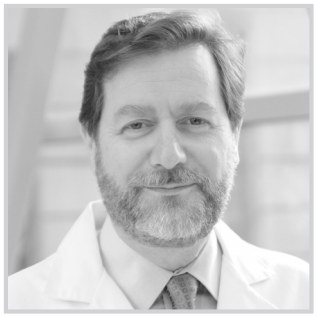
"These programmes endeavour to mitigate the lack of expensive diagnostics and therapies by teaching local doctors to make the best use of available drugs and basic diagnostic techniques."

Q8 Being the chair of such an important committee is obviously a great deal of workload to add onto one's schedule. Why do you think it is important to be a part of such a committee?

I think it is important to be part of the International Members Committee to ensure that aspects of haematology of global interest and relevance are addressed. It has been a great privilege to lead this committee and all the hard work has been very rewarding. The international footprint of ASH has expanded rapidly over the past few years, and numerous exciting new initiatives have been launched that will ultimately benefit research and patient care globally, which emphasises the mission of ASH in "helping haematologists conquer blood diseases worldwide."

Q9 What is the future of haematology as a specialty, and what are the largest problems that we will have to overcome?

I think haematology has a bright future since there is a wide spectrum and high burden of encompassing haematological diseases, and new therapies are emerging every year. Especially because of the readily accessible nature of blood and bone marrow samples, this is an exciting research discipline that facilitates major discoveries.



Dr Pierluigi Porcu

Chair of the ASH Foundation Committee
Sidney Kimmel Cancer Center, Philadelphia, Pennsylvania, USA

Q1 Can you please briefly explain how you came to become involved with the ASH Foundation Committee, and what its mission is?

I have been a member of ASH since I became a fellow in 1996, and it has been instrumental in supporting my career development as a haematologic malignancies researcher. ASH encourages all its members to engage in the activities of the society, become volunteers, and contribute to the overall mission, which is helping haematologists to conquer blood diseases worldwide. This can be done in multiple ways, one of which is to serve on committees and task forces. I became a member of the ASH Foundation Committee in 2014, and in 2016 I was appointed Committee Chair, a position I have held until this year. I am now stepping down as Chair and I will be serving ASH in other volunteer capacities.

The mission of the ASH Foundation Committee is publicly available on the ASH website, but briefly, the ASH Foundation Committee is responsible for collaborating with the Director of the ASH Foundation and ASH's Executive Director to maximise charitable support from individual donors, foundations, and corporations. The Foundation's mission is to move haematology forward through charitable support for ASH programmes and to help haematologists conquer blood diseases worldwide. As Committee Chair, I was gratified to see increases in the number of research and training awards, publications, and global programmes that would not have been possible without the funds raised by the ASH Foundation.

Q2 You presented at this year's meeting a session on a combination therapy for Hodgkin lymphoma and T/NK cell lymphoma. Can you tell us a bit more about these findings?

This year I presented the Phase Ib data on an ongoing Phase Ib/II study of a new oral therapy for patients with Epstein–Barr virus (EBV)-positive relapsed/refractory lymphomas.[1] The clinical trial combines orally administered nanatinostat (Nstat), a novel Class I histone deacetylase inhibitor (HDACi), with the known oral antiviral drug valganciclovir (VGCV). EBV-positive lymphomas are typically aggressive and have poor outcomes, with few exceptions. The rationale for this combination is that Nstat induces the expression of an EBV-encoded protein called BGLF4 that can activate VGCV, which then acts as a cytotoxic drug in EBV-infected cells. While the exact number of EBV-positive lymphomas in non-immunocompromised patients is not known because the presence of EBV is not routinely tested for in all lymphomas, we estimate that a significant fraction, possibly as high as 15–20%, of aggressive B-cell and T/NK-cell non-Hodgkin's lymphomas (NHL) and 20–30% of Hodgkin's lymphomas are EBV-associated.

Until now, with the exception of adoptive cell therapy strategies, no EBV-targeting therapy has been in clinical trials for this patient population. Our study evaluated the combination of Nstat and VGCV across three dose escalation cohorts. The combination was well-tolerated and the most common serious adverse events (AE) were haematologic; all were resolved without sequelae. No patients discontinued therapy due to a treatment-related AE. The most frequent treatment-related Grade 3–4 adverse events were thrombocytopenia (25%), neutropenia (20%), and anaemia (10%). All patients dosed at the recommended Phase II dose, including

additional Phase IIa patients, had no Grade 3–4 haematologic AE. We observed responses across all doses, and in all lymphoma subtypes, including HDACi-refractory. We were able to determine the recommended Phase II dose of the combination, and the Phase IIa dose expansion portion of the study is now ongoing, with responses and no new severe adverse events. The overall response rate in the Phase Ib cohort was 56% (10/18), with 28% (5/18) complete response, and a clinical benefit rate (the sum of overall response rate plus stable disease rate) of 78% (14/18), with a median duration of treatment for responders of 6.5 months. Two responders remained on treatment for over 12.0 months, including one patient who remains in complete response at 17.0 months following 12.7 months on treatment. So, we are excited about the early results of this clinical trial, we are encouraged by what we are seeing in the Phase II part, and we are looking for a few additional participating institutions.

Q3 Given your extensive experience in lymphoma education and mentoring, is it a safe assumption to make that these are both vital aspects of your working career?

One of the most rewarding aspects of working in an academic environment, such as the Thomas Jefferson University, Philadelphia, Pennsylvania, USA, where the research and education missions are on par with patient care, is the interaction with students and trainees; the ability to work with bright, young fellows and junior faculty, and observe as they learn the basics, become confident, set their career goals, and then move on to establish themselves as independent investigators.

Mentoring is something that requires continuous and frequent interactions; the ability to give and take honest feedback, and a mutually agreed commitment to academic excellence and scientific rigor. You can talk these values, but you can only apply and teach them by example. The learning is often mutual, and I cannot think of a situation in which I did not learn something valuable myself from mentoring someone. The challenge these days is that the time that clinical faculty have to spend on mentoring and teaching is less and less, because in most institutions teaching is not valued as much as clinical effort, and mentoring is an activity that does not bring revenue and for which the return on investment is non-monetary and typically deferred. It takes visionary and strong leadership to support the academic mission, and that is not always available at all institutions.

Q4 You have spoken before of patient empowerment being an overarching principle of yours. What is your strategy for best achieving this empowerment? Are patients always equipped to process this information?

One of my strongest convictions, based on 20 years of empirical observations in the clinic, is that very few patients do not want to be active participants in their own care, particularly when they face an illness such as cancer, with its potentially devastating impact on themselves and their families. The guiding principle of my team at Jefferson is patient empowerment, based on knowledge. The more people know about their cancer, the more prepared they are for the journey, including how to face adversities and challenges along the way. Knowledge also grounds hope in facts; one of our foremost goals as oncologists must be to find ways to help patients and caregivers to discover how they can be part of the team and how they can be part of the solution. This is sometimes easier said than done, because every patient brings to the table a different set of experiences, educational backgrounds, personal beliefs, family or group traditions, emotions, and deep personal convictions that influence their perception of the reality they live in and the goals that they wish to achieve, sometimes irrespective of what is realistically doable. So, what is now often called cultural competency, and in the past was called bedside manners or human touch, is an absolutely indispensable core competency to be able to take care of patients with cancer.

In the Division of Hematologic Malignancies and Hematopoietic Stem Cell Transplantation at Jefferson, we have initiated a major research effort, in collaboration with our population science colleagues, to learn how to be effective communicators and how to productively interact with patients with blood cancer, in ways that are personalised and specific for the cancer type

that each patient has. For example, we have an ongoing project in T-cell lymphoma, which is a rare type of lymphoma for which there is very little information.

Q5 You recently published a review on cutaneous T-cell lymphoma, specifically advances in disease diagnosis. Can you summarise some of the take-home messages of this paper?

Cutaneous T-cell lymphomas (CTCL) continue to represent a significant diagnostic challenge, particularly in early stage, although technological innovations, such as high throughput sequencing of the T-cell receptor (TCR), are now being introduced in the diagnostic work up that are very likely to lead to significant advances in care, including early diagnosis. Still, resolving a difficult diagnostic dilemma remains an everyday challenge for oncologists and dermatologists, which involves synthesising varied clinical presentations with complex immunohistochemical findings as well as molecular data and ancillary testing, such as flow cytometry and standard TCR gene rearrangement analysis. The field is also ever changing, with new diagnostic and prognostic considerations flooding the literature, making sorting through the latest developments seem almost impossible.

In 2017, the World Health Organization (WHO) published a major revision of the fourth edition of the classification of haematopoietic and lymphoid neoplasms and introduced several changes to the current classification and understanding of CTCL. Many of the subtypes described in the latest WHO classification are quite rare and may not be routinely encountered, thus a basic knowledge of the categories is the first step to diagnosis.

One of the key elements of a successful diagnosis of CTCL is a strong familiarity with all its clinical manifestations because a skin biopsy often cannot be adequately interpreted without knowing the clinical context in which it was obtained, which should include the visual inspection of the lesions. Another key element is not to rely too heavily, or exclusively, on molecular analyses such as TCR gene rearrangement analysis. Too often we see skin biopsy reports deferred and being held hostage to the TCR gene rearrangement analysis, often delayed because it is done at reference labs. Thirdly, be watchful for CTCL subtypes that may be difficult to distinguish from systemic, non-cutaneous T-cell neoplasms, such as ALK1-negative anaplastic large cell lymphoma (ALCL), mature T-cell leukaemias, and NK cell lymphomas. Finally, be aware of the exceptions that prove the rule. For example, the ruling dogma is that primary cutaneous ALCL is always ALK1 negative, which is almost always true. However, there are rare cases of ALK1-positive ALCL, particularly in young patients that are clearly skin limited, and therefore fall under the classification of primary cutaneous ALCL.

Q6 Finally, with the continued development of exciting immunotherapies, this is a very exciting time for the haematological field. What areas of research are you most anticipating heading into the new year?

The immune-oncology revolution is clearly one of the most inspiring scientific tales of the last decade. I believe that in the next year we will be learning much more about how to combine different immune-oncology drugs or different immunotherapy modalities, not limited to the canonical checkpoint inhibitors such as PD-1 and PD-L1/2. A number of drugs targeting other immune checkpoints, such as TIGIT, TIM-3, CD47, VISTA, KIR, just to name a few, are in clinical trials.

In addition, I am very excited about a number of new bispecific, and tri-specific, antibodies that combine a tumour cell engager moiety with an effector cell engager. And now a new class of sophisticated multi-specific antibodies that simultaneously bring activated effector cells in close proximity to the tumour cell, while inhibiting co-inhibitory signals, are in development. We will also be learning more on cellular therapy with T cells or NK cells that carry new chimeric receptors. Finally, we will start learning more about mechanisms of resistance to immuno-oncology drugs and how to overcome them with new strategies.

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Abstract Reviews

The following pages showcase abstract reviews presented by the authors themselves at this year's ASH Meeting

Fli-1 Transcriptionally Integrates Microenvironmental Cues Sensing by Self-Renewing Haematopoietic Stem and Progenitor Cells

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Keywords: Cellular sensing, endothelial, *ex vivo* expansion, Fli-1, haematopoietic stem cells, microenvironment, niche, self-renewal, transcriptional regulation.

Citation: EMJ Hematol US. 2020;1[1]:32-34. Abstract Review No: AR1.

INTRODUCTION

During adulthood and embryogenesis, fate decisions of haematopoietic stem and progenitor cells (HSPC), such as specification, self-renewal, and differentiation, are tightly regulated by their neighbouring niche cells.[1] Moreover, distinct types of niches supply differential cues to direct alternative cell fates for HSPC.[2,3] Currently however, the intrinsic mechanisms balancing HSPC response obliqueness to microenvironmental signals are unknown.

Fli-1 is an E26 transformation specific transcription factor expressed by vascular beds and haematopoietic lineages. Fli-1 belongs to the family of 'heptad factors' which are hypothesised to specify and sustain a haematopoietic cell fate.[4,5] While Fli-1 overexpression is linked to leukaemia,[6]

the functional role Fli-1 plays in HSPC specification and maintenance remains undefined.

METHOD AND RESULTS

The authors showed that inducible global deletion of Fli-1 using a Cre/lox model (Fli-1$^{ROSA\Delta}$) results in a rapid thrombocytopenia-associated mortality. Transplantation of Fli-1$^{ROSA\Delta}$ bone marrow cells into wild-type (WT) recipients, to exclude vascular-mediated defects, followed by induction of Fli-1 deletion resulted in the same phenotype. In a set of modulated competitive transplantation experiments (differential induction time points pre or post-transplant), the authors observed defective ability of Fli-1$^{ROSA\Delta}$ HSPC to lodge, engraft, and to sustain haematopoiesis post-repopulation. Fli-1-deficient HSPC exhibit reduced quiescence, a hallmark of stemness, and display enhanced apoptosis. Thus, Fli-1 is essential for previously unrecognised cell-autonomous HSPC functions.

To determine whether Fli-1 deletion abrogates haematopoietic specification, Fli-1 was conditionally deleted using a developmental haematopoietic Cre/lox model (Fli-1$^{Vav\text{-}1\Delta}$) which resulted in premature mortality. Reduced presence of embryonic Fli-1$^{Vav\text{-}1\Delta}$ liver HSPC was observed at e12.5. Moreover, using a developmental endothelial (CDH5)-Cre/lox model (Fli-1$^{CDH5\Delta}$), the authors observed that reduced numbers of haematopoietic cells were still detected in the aorta-gonad-mesonephros (AGM) region. Two *in vitro* co-culture systems were also applied to study Fli-1 in the endothelial-to-haematopoietic transition. First, isolated haemogenic endothelial cells from WT and Fli-1$^{ROSA\Delta}$ embryos were cocultured with AGM-derived vascular niche.[7]

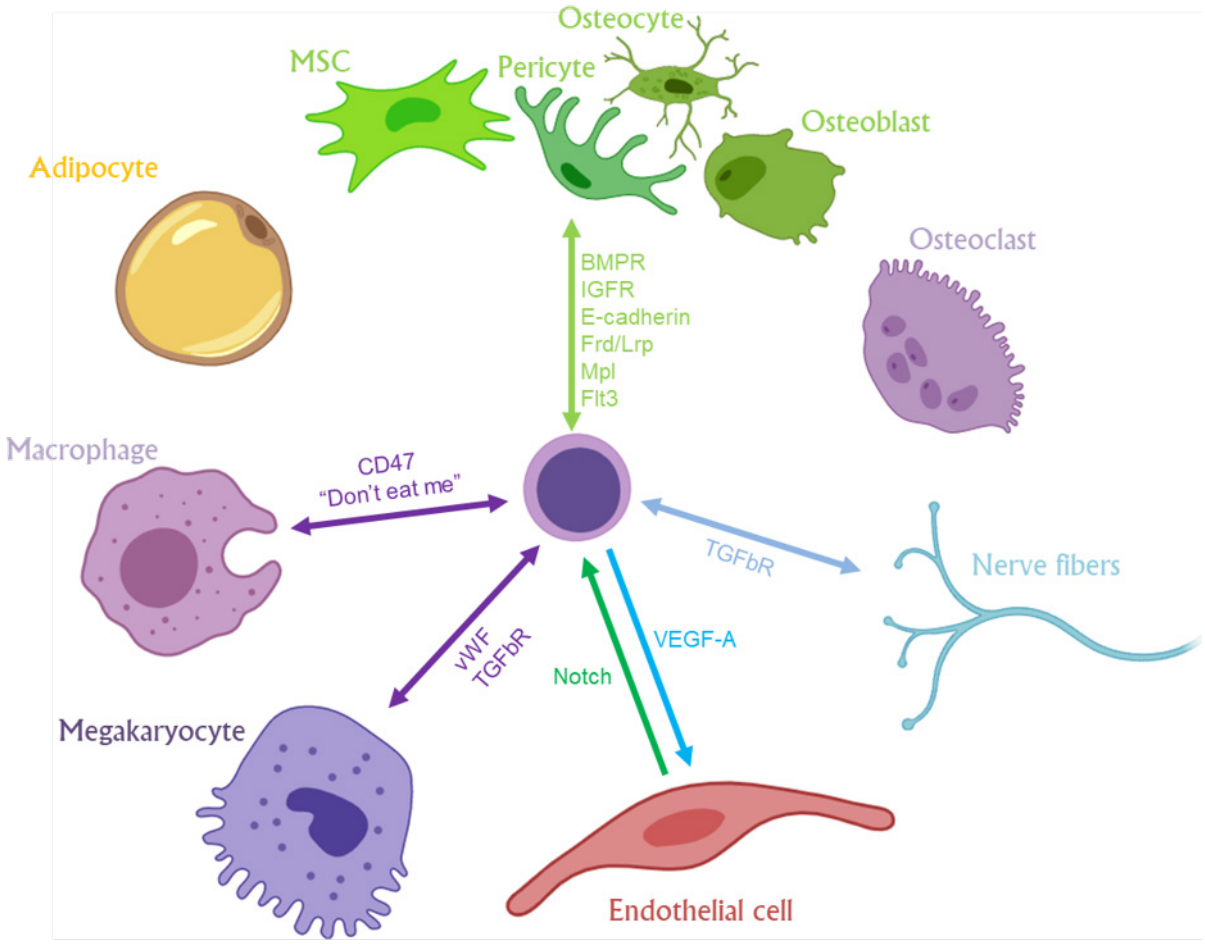

Figure 1: A summary model presenting Fli-1-regulated sensory elements that may mediate the interaction and crosstalk between haematopoietic stem and progenitor cells and their diverse neighbouring niche cells in the bone marrow microenvironment.
MSC: mesenchymal stromal cell.

Haemogenic endothelial cells isolated from Fli-1$^{ROSA\Delta}$ AGM were still able to convert to CD45+ cells, however these cells did not expand on a vascular niche. Secondly, an endothelial-to-haematopoietic reprogramming system was implemented in which isolated lung endothelial cells were virally introduced with DOX inducible FosB, Gfi1, Runx1, and Spi1 (FGRS) factors and co-cultured with vascular niche cells.[8,9] Both WT and Fli-1$^{ROSA\Delta}$ endothelial cells were able to acquire a haemogenic-like state resulting in a final capacity to convert into haematopoietic cells. Again, Fli-1$^{ROSA\Delta}$ cells displayed fewer numbers of CD45+ cells at the end point, presumably due to impaired interaction with the vascular niche. Induction of Fli-1 deletion *in vitro* in adult HSPC revealed loss of dependency on vascular niche inductive signals, as no additive expansion effect was observed for Fli-1$^{ROSA\Delta}$ HSPC in the presence of a vascular niche. Hence, Fli-1 is not essential for haematopoietic specification but rather essential for HSPC expansion and self-renewal.

Differential RNA-seq analysis combined with epigenetic studies of expanding WT and Fli-1$^{ROSA\Delta}$ HSPC revealed dysregulation of Fli-1-controlled pathways involved in transduction of microenvironmental signals for self-renewal. Focusing on HSPC-vascular niche interaction, this study observed dysregulation in Notch and VEGF pathway elements that mediate an HSPC-vascular niche crosstalk.[10]

CONCLUSION

Decrypting the mechanism(s) by which Fli-1 orchestrates HSPC self-renewal may promote an improved expansion protocol of human HSPC pre-transplantation and provide additional insights for microenvironmental sensing by Fli-1-dependent normal and leukaemic cells (Figure 1).

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Novel CD33 Antibodies Reveal CD33 Splicing Polymorphism Variant Localisation and Therapeutic Implications

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Keywords: Acute myeloid leukaemia (AML), CD33, immunotherapy, personalised medicine, targeted therapy.

Citation: EMJ Hematol US. 2020;1[1]:35-36. Abstract Review No: AR2.

INTRODUCTION

Acute myeloid leukaemia (AML) is a heterogeneous haematologic malignancy characterised by dysfunctional proliferation and differentiation of myeloid progenitor cells.[1] Though the standard 7+3 treatment regimen, consisting of cytarabine, daunorubicin, and etoposide induces remission, a significant proportion of patients relapse resulting in negative outcomes overall.[2]

To address the need for new effective agents to treat AML, several immunotherapeutic therapies are being investigated in various AML treatment regimens. CD33 is a myeloid cell surface protein present on leukaemic cells in >85% of AML patients and consists of 2 extracellular domains, the IgV and IgC domains, as well as an intracellular immunoreceptor tyrosine-based inhibition motif (ITIM).[3] CD33 internalisation upon antibody binding makes it a lucrative target in AML with treatment strategies including antibody-drug conjugates, such as gemtuzumab ozogamicin (GO), an antibody–drug conjugate reapproved in 2017 for treatment of AML, and other immunotherapeutics.[4-6] While CD33-directed therapies have revolutionised AML treatment strategies, studies have shown evidence of interpatient variation in CD33-directed therapeutic response.[7] In particular, the authors of this article previously reported occurrence of a splicing single nucleotide polymorphism, *rs12459419* (C>T), which resulted in skipping of exon 2 and formation of a shorter CD33 isoform (CD33D2) lacking the IgV domain.[8] Given that all currently available CD33-directed therapies and immunophenotyping diagnostics are based on recognition of the IgV domain, the minor allele of *rs12459419* is associated with lower cell surface levels of CD33 and altered response to GO. Consequentially, patients with at least one variant allele do not benefit from inclusion of GO in their chemotherapeutic regimen.[8] The lack of benefit observed in heterozygous patients remains an enigma and warrants in-depth investigations into CD33D2 biology. Thus, the aim of this study was to develop novel antibodies which recognise CD33D2 and utilise these antibodies to characterise the expression pattern, localisation, and therapeutic implications of CD33D2, including its potential as a novel drug target and role in the compromised response to GO observed in heterozygous patients.

METHODS

Novel CD33 antibodies were developed by immunising Balb/c or C57BL/6 mice with peptides spanning the IgC domain. The novel antibodies, 5C11-2 and HL2541, were then confirmed for recognition of CD33 by Western blotting using the CD33-Ba/F3 cell line engineered to express either CD33FL or CD33D2. Subsequently, immunofluorescence assays using the novel antibodies and the IgV targeting antibody, P67.6, were performed to assess the expression pattern and localisation of CD33 isoforms using the Ba/F3 cell line system, AML cell lines of different *rs12459419* genotypes, and primary AML bone marrow specimens.

RESULTS

Western blotting analysis using the novel IgC antibodies and the ITIM domain-directed antibody, E6, as a control, confirmed recognition of both CD33FL and CD33D2 by the novel IgC-directed antibodies; however, only CD33D2 was recognised on the cell surface in flow cytometric assays. These results indicate a potential steric hindrance caused the clustered extracellular Ig-folds which may stifle recognition of CD33FL by IgC-targeting antibodies. Flow cytometry performed on AML cell lines stained with P67.6, 5C11-2, and HL2541 revealed extracellular and intracellular recognition CD33FL while CD33D2 was only recognised intracellularly regardless of genotype. Ongoing work continues using primary AML specimens to fully elucidate the cell surface expression of CD33D2 and the potential for its targeting. Once confirmed, these results hold potential for establishing CD33 biology and understanding the associated therapeutic implications.

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Safety and Efficacy of 1,020 mg Total Dose Infusion of Ferumoxytol in a Veterans Health System

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BACKGROUND

Ferumoxytol is an intravenous (IV) formulation of iron that can be infused quickly, making it a convenient choice. It is typically given in 2 doses of 510 mg. Auerbach et al.[1] previously described utilising a single dose of 1,020 mg over 15 minutes safely and effectively. A total dose infusion of 1,020 mg has been described by experts in the field as the "maximum safe dose."[2,3] There exists very little published literature about the safety and efficacy of this off-label dosing; however, it is an attractive administration schedule due to convenience of the one-time dose. In July 2018, the authors began to administer a single 1,020 mg dose of ferumoxytol to patients diagnosed with iron deficiency at the north Florida/south Georgia Veterans Health System in Gainesville, Florida, USA. The purpose of this review is to evaluate the impact of the use of the 1,020 mg ferumoxytol dose to ensure safe, effective, and efficient utilisation in the management of iron deficiency anaemia.

METHODS AND RESULTS

A retrospective chart review was conducted on patients who received ferumoxytol from February 1st 2018 to January 31st 2019 to capture approximately 6 months of data prior to, and after, the dosing strategy change. Patients were excluded from review if they had received IV iron within 3 months prior to the study period. Parameters collected included pre and post haemoglobin, iron saturation and ferritin concentrations, dose of iron, frequency and number of infusions, post infusion monitoring time, and hypersensitivity reactions. The primary outcome was assessing safety, particularly the rate of infusion reactions for the entire cohort of patients. Secondary outcomes included efficacy and clinic utilisation. Number of visits, baseline and change in haemoglobin, ferritin and iron saturation following 1 dose of 1,020 mg, or 2 doses of 510 mg were compared using paired t-tests. Rate of infusion reactions was compared between all patients who received either dose using Fisher's exact test.

A total of 212 patients were screened and 140 included in the analysis. During the study period, 270 total doses of iron were given. Fifty-nine patients (42%) received only 510 mg doses and 60 (43%) received only 1,020mg doses and were included in the efficacy analysis. An additional 21 (15%) received both 510 mg and 1,020 mg doses and were included in the analysis of reaction rate. Baseline characteristics were similar between the groups (Table 1). Response to iron infusions were not significantly different between the dosing strategies. Mean change in haemoglobin was 1.96 g/dL for the 510 mg group and 2.00 g/dL for the 1,020 mg group (p=0.726). Mean change in ferritin was 114 ng/mL and 120 ng/mL (p=0.8203). Likewise, mean change in iron saturation was 13.6% and 14.3% (p=0.7808). The rate of infusion reactions was not increased with the higher dose, with only 1 reaction occurring in each group (0.57% and 1.04%; p=1.00). Both infusion reactions were able to be treated on an outpatient basis and the patients were discharged from the infusion clinic on the same day. Administering the 1,020 mg dose significantly reduced the number of infusion room visits required, with an average of 2 visits for 510 mg patients and 1 visit for 1,020 mg patients (p<0.0001).

CONCLUSION

In conclusion, implementation of a total dose infusion of 1,020 mg of ferumoxytol reduced the number of infusion room visits without increasing infusion reactions or compromising efficacy. This strategy could be considered at other institutions to improve infusion room access, patient convenience, and reduce costs.

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Table 1: Comparison of patients treated with ferumoxytol 510mg and ferumoxytol 1,020 mg.

Patient characteristic	510 mg (n=59) mean (range)	1,020 mg (n=60) mean (range)	Significance (p value)
Baseline haemoglobin (g/dL)	9.83 (6.7—14.6)	9.74 (6.7—13.3)	0.726
Change in haemoglobin (g/dL)	1.96 (-0.8—5.9)	2 (-1—5.7)	0.7639
Baseline ferritin (ng/mL)	38.2 (3—415)	26.2 (4—417)	0.2923
Change in ferritin (ng/mL)	114 (-92—496)	120 (-8—1012)	0.8203
Baseline iron saturation (%)	8.95 (3—28)	11.37 (3—78)	0.2155
Change in iron saturation (%)	13.6 (-14—47)	14.3 (-49—173)	0.7808
Infusion visits per patient (#)	2.05 (1—6)	1.10 (1—2)	<0.0001

Assessment of Mortality and its Associated Risk Factors in Patients with Transfusion-Dependent Thalassaemia in India

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India is home to approximately 100,000 people living with transfusion-dependent severe thalassaemia,[1] and yet very little data on survival are available for this group of patients. Since the year 2000, oral chelators, namely deferasirox and deferiprone, have been widely available, and universal screening of blood units for HIV, hepatitis B, and hepatitis C has been mandated by the Indian government. In this era, which has opened the possibility of safer blood and access to iron chelation therapy, one would expect to see improvement in the outcomes for these patients, thereby furthering the case to have benchmarks of mortality associated with thalassaemia. A group led by the Sankalp India Foundation, Bangalore, India, has recently summarised life expectancy and risk factors of death associated with transfusion-dependent thalassaemia in 1,087 patients from 5 centres in South India from 2011 to 2018, and presented this data at the 2019 American Society of Hematology (ASH) Annual Meeting in Orlando, Florida, USA.[2] The study concluded that 50% of transfusion-dependent thalassaemia patients who are receiving supportive care die by the age of 26 years old (Figure 1). A significant finding included the fact that the mortality rate of those <5 years old was also 7.0-times higher than that of the general population.

The study also analysed the potential contributors to mortality. Among the factors which were reported to be significant, patients with transfusion-transmitted infections were reported to be at a 3.4-times higher risk of death. Patients who had high serum ferritin levels (>4,000 ng/dL) were at a 4.6-times higher risk of death compared to those who had serum ferritin under control (<1,000 ng/dL). Patients who had a haemoglobin drop of >2.2 gm/dL every week were at a 7.7-times higher risk of death, compared to those who dropped ≤1 gm/dL every week. The study showed no significant differences between sexes, economic status, and the distance travelled to receive care, which is consistent with previous reports.[4] The study also showed that as patients received more years of proper care and management in dedicated centres, mortality rates dropped significantly.

This study offers baseline data and methodology to assess survival associated with thalassaemia based on real-world data. The findings may have major implications on care planning and delivery for thalassaemia patients, and suggests that optimising blood transfusion, intensifying chelation, and preventing transfusion-transmitted infection continue to be particularly important.

Moreover, as an increasing number of centres in India offer cure through bone marrow transplantation directly,[5] it seems of paramount

importance to have some objective data guiding patient and family counselling. Sustained efforts in these areas, coupled with increased prevention and access to safe bone marrow transplant, will ease the burden for both families and public healthcare.

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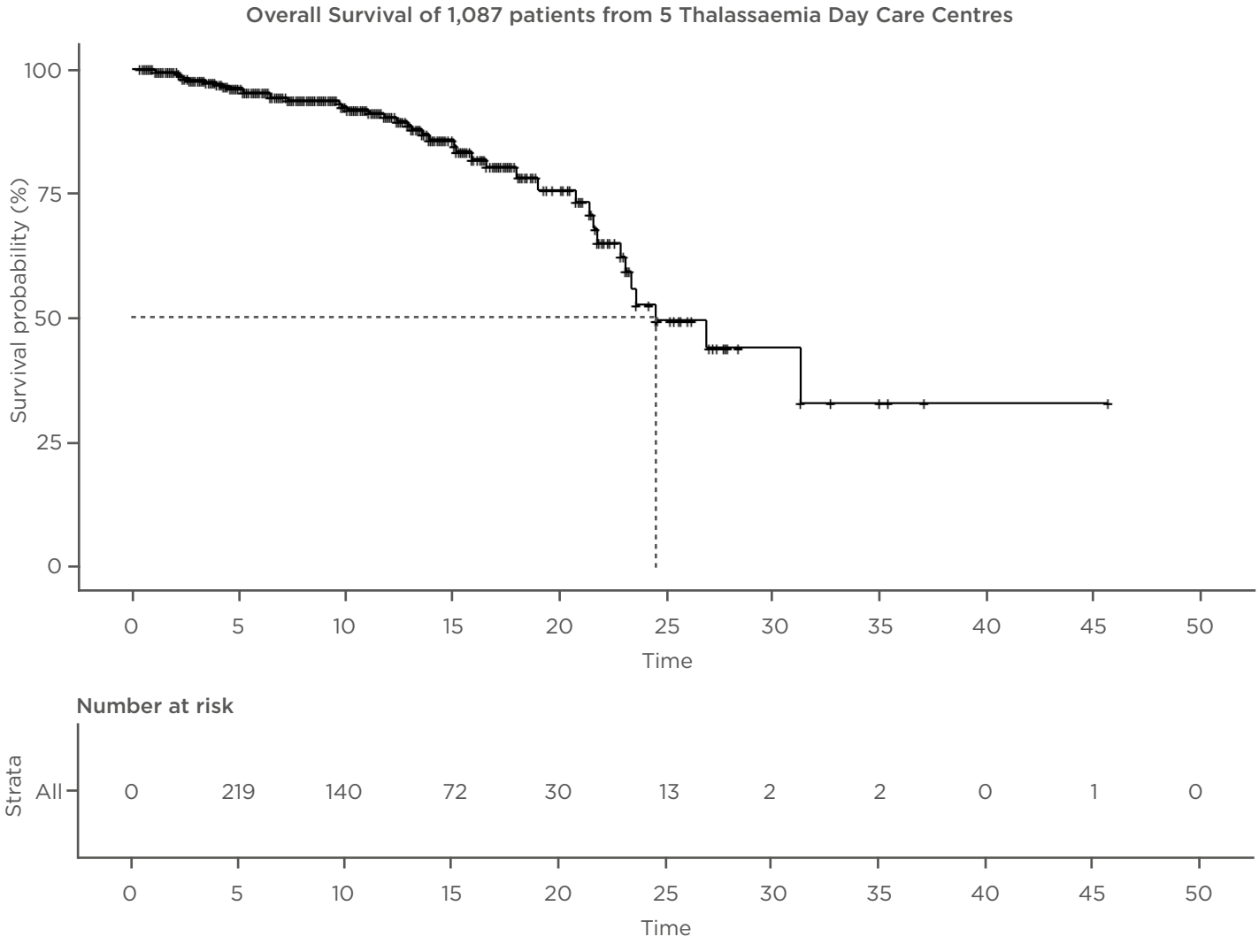

Figure 1: Kaplan-Meier survival curve.

The *Cnaan and Ryan*[3] approach was employed, whereby patients enter and leave the study cohort and observation begins only at enrolment, and not at the onset of the disease.

Lactate Release by Inflammatory Bone Marrow Neutrophils Induces their Mobilisation via Endothelial GPR81 Signalling

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Keywords: Bone marrow endothelial cells, immune-metabolism, infection, inflammation, lactate, lipopolysaccharide, neutrophils.

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BACKGROUND

Innate immune neutrophils provide the first line of host defence against bacterial infections. The energy required for neutrophil functions is derived primarily from glycolysis, of which a key product is lactate. Elevated lactate levels, coinciding with a rise of neutrophils in the circulation and inflamed tissues, are associated with pathological conditions including shock, sepsis, and ischaemia.[1-3]

Lactate is also released by human neutrophils,[4] and it has been shown that enhanced glycolysis promotes key neutrophil functions including phagocytic activity[5] and neutrophil extracellular trap formation.[6,7] However, the mechanisms by which glycolysis is triggered in neutrophils and contributes to their recruitment and function in acute inflammation is poorly understood.

METHODS AND RESULTS

Utilising murine bacterial infection models, the results of this study reveal that neutrophil-derived lactate promotes their mobilisation from the bone marrow (BM) to the circulation.[8]

RNA-seq of sorted BM neutrophils documented that bacterial lipopolysaccharide (LPS)-activated neutrophils upregulated enzymes catalysing the first part of glycolysis and downregulated the expression of tricarboxylic acid (TCA) cycle enzymatic genes. In addition, LPS treatment increased NADPH-oxidase (NOX)-mediated reactive oxygen species and HIF-1α levels in BM neutrophils, triggering lactate production and release. $NOX^{-/-}$ and myeloid specific HIF-1α-deficient mice failed to elevate lactate production in the BM during acute inflammation. $NOX^{-/-}$ mice failed to mobilise activated high levels of reactive oxygen species (ROS^{high}) neutrophils in response to LPS, while lactate administration partially rescued this defect.

Further deciphering the mechanisms of lactate-induced neutrophil mobilisation, the authors identified for the first time that BM endothelial cells abundantly express the highly selective lactate G protein-coupled receptor 81 (GPR81). Lactate treatment reduces vascular endothelial-cadherin expression in the BM sinusoid endothelial cells and increases BM vascular permeability via endothelial GPR81 signaling. $GPR81^{-/-}$ mice failed to mobilise neutrophils in response to LPS unless rescued by vascular endothelial-cadherin disrupting antibodies.

In addition to its direct effect on BM vascular permeability, lactate also acts on BM neutrophil mobilisation by elevating the neutrophil mobilising chemokines CXCL1 and CXCL2 in both BM and blood. CXCL1 and CXCL2 are ligands of the CXCR2 receptor involved in neutrophil mobilisation.[9] The study found that lactate-induced elevation of CXCL1, unlike lactate-enhanced vascular permeability, is GPR81-independent, indicating that this metabolite may drive neutrophil mobilisation via multiple pathways.

Finally, to examine the potential clinical relevance of the findings, the authors infected wild type (WT), $NOX^{-/-}$, and $GPR81^{-/-}$ mice with *Salmonella typhimurium* and found that this pathogen drove

high generation of ROS, elevated HIF-1α levels, and triggered lactate production and release in WT BM neutrophils. In contrast, BM neutrophils of infected $NOX^{-/-}$ mice exhibited significantly lower HIF-1α and impaired lactate production and release. Consequently, WT mice infected with *S. typhimurium* had higher levels of neutrophils in the blood, compared to their $NOX^{-/-}$ or $GPR81^{-/-}$ mice counterparts. Altogether, the data reveals that the same regulatory mechanisms by which neutrophils respond to LPS challenges are used during bacterial infection with *S. typhimurium.*

CONCLUSION

The study showed the first example of a released neutrophil metabolite acting as a potential target for modulating neutrophil mobilisation to peripheral organs exposed to acute infections. Thus, understanding metabolic checkpoints in BM neutrophils suggests new perspectives to develop treatments that target unregulated inflammatory neutrophils during bacterial infection.

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Acute Chest Syndrome in Children with Sickle Cell Disease: Current Perspectives on Pathogenesis and Treatment

EDITOR'S PICK

Sickle cell disease is a severe haemoglobinopathy with highly variable clinical features. Acute chest syndrome is one of its most severe complications in regard to mortality and morbidity, and heavily impacts on quality of life. The pathophysiology of this condition is not completely understood, and its rapid recognition and immediate treatment are essential but, unfortunately, not yet fully implemented. This very well written and comprehensive article by Uwaezuoke helps to clarify and diffuse the knowledge of this extremely dangerous complication that can involve both children and adults affected by the disease.

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Abstract

Acute chest syndrome (ACS) is the most prominent cause of mortality in children with sickle cell disease. Its cause was initially not clearly understood, but there are now established concepts regarding its aetiopathogenesis. This narrative review discusses the current perspectives on sickle cell disease pathogenesis and treatment. The PubMed database was searched for articles that met the review objective. The major causative factors are pulmonary infections, pulmonary infarction, and pulmonary fat embolism from bone marrow necrosis. These factors initiate events that result in ACS, in which a vicious cycle of infarction, inflammation, and lung collapse occurs, leading to ventilation-perfusion mismatch and hypoxaemia. ACS is best managed in hospital settings because intensive care of the patient may be required. Despite its complex management, the primary treatment modalities are supportive care, transfusion therapy, and pharmacotherapy. Although the efficacy of several modalities in attenuating or preventing ACS are well established, the outcomes from instituting others are not convincing. More research is, therefore, needed to strengthen the evidence for their therapeutic efficacy.

INTRODUCTION

Acute chest syndrome (ACS) is an acute inflammatory lung injury that usually follows the vaso-occlusive crisis (VOC) seen in sickle cell disease (SCD). It is defined by the presence of a new pulmonary infiltrate on a chest radiograph, in association with fever and respiratory symptoms or signs such as cough, chest pain, hypoxaemia, wheezing, and fast breathing.[1-3] It is the most prominent cause of mortality in children with SCD, occurring in >50% of patients who are admitted with VOC.[4] A recent study identified young age, male sex, asthma comorbidity, and obstructive sleep apnoea as significant risk factors for ACS.[5] Additionally, young age and a history of asthma are associated with recurrence of ACS.[6] Risk factors and symptoms of both asthma flare-ups and ACS are remarkably similar.[7] Nevertheless, a multicentre prospective study observed that older patients (aged ≥20 years) experienced a more severe clinical course than their younger counterparts.[8]

Although the pathogenesis of the syndrome was thought to be mostly idiopathic, there are now established concepts about its aetiopathogenesis. For instance, a recent narrative review indicated that the aetiologic factors consist of pulmonary infections, pulmonary infarction, and pulmonary fat embolism, which originate from bone marrow necrosis.[9] The underlying pathogenic mechanism involves a hypoxia-induced increase in intercellular adhesion of sickled red blood cells and their increased adhesiveness to the endothelial lining of the pulmonary vasculature. This is facilitated by the interaction between erythrocytic very late activation antigen-4 and endothelial vascular cell adhesion molecule-1 (VCAM-1).[10] The resultant vaso-occlusion initiates a vicious cycle of pulmonary infarction, inflammation, and lung collapse. The clinical deterioration in ACS is specifically caused by the initial hypoxia-induced upregulation of pulmonary endothelial VCAM-1, which is not neutralised by the reactive formation of cytoprotective mediators such as nitric oxide (NO); this leads to intrapulmonary adhesion.[10]

A clear understanding of the causes and pathogenesis of ACS and applying appropriate therapeutic interventions may prevent fatal outcomes. The present narrative review discusses the current perspectives on the pathogenesis and treatment of this syndrome. It presents a discourse on the effective modalities for managing ACS in children with SCD.

LITERATURE SEARCH: STRATEGY AND OUTCOME

A search of the PubMed database using the descriptors "acute chest syndrome" and "sickle cell disease" yielded 931 published articles. Addition of search terms such as "children" and "pathogenesis and treatment" scaled down the number of articles to 539 and 288, respectively. After exclusion of duplicates, case reports, items unrelated to the review objective, and letters to the editor, 102 original and review articles (published in the English language) were selected. Additionally, the items that met the inclusion criteria for review consisted of those published from the previous four decades to the current date. Relevant information was extracted from these papers for the present narrative review.

RISK FACTORS FOR ACUTE CHEST SYNDROME

Several modifiable and nonmodifiable risk factors of ACS have been identified from numerous studies. These risk factors include young age,[5,7,11] SCD genotypes such as HbSS or HbS-ß^0 thalassaemia,[11,12] asthma comorbidity,[6,13-17] low proportion of fetal haemoglobin,[11] high steady-state haemoglobin concentration,[11] high steady-state leukocyte count,[11,13,18] primary or secondary tobacco smoke exposure,[19-21] more than three episodes of VOC in the previous year,[13] and recent surgical intervention.[22]

Some of these factors can predict the occurrence of ACS based on the following possible mechanisms. Firstly, high asthma prevalence rates in children with SCD[14,23] underscore the vital role of asthma in the development of ACS. Several studies have noted a direct relationship between asthma flare-ups and episodes of ACS at a young age.[16,17,24-26] It does appear that children with SCD and comorbid asthma are very likely to develop respiratory symptoms during a VOC.[25] The suggested mechanisms for the predisposition to ACS include a damaged vasculature, bronchospasm-induced hypoxia

leading to increased red blood cell sickling and subsequent vaso-occlusion, as well as the inflammation arising from disease synergy. These mechanisms contribute to and accentuate the vicious cycle of pulmonary infarction, inflammation, and lung collapse, which characterise ACS. Secondly, the high steady-state haemoglobin concentration and leukocyte count associated with ACS risk are thought to be contributory factors through their alteration of blood rheology. The increased blood viscosity promotes vaso-occlusive events. However, after development of VOC, acute haemolysis may contribute to the evolution of pulmonary injury through elevated amounts of free circulating haem. This product of haemolysis contributes to the development of ACS in paediatric patients with SCD.[27] Its role in the pathogenesis of the syndrome has been highlighted subsequently in this review. Finally, ACS triggered by secondary exposure to tobacco smoke is explained by mechanisms such as lower airway obstruction in children with SCD, as well as increased expression of VCAM-1 with airway inflammatory response.[28]

Notably, there are clear differences between the clinical presentation of ACS seen in children and adults with SCD. Whereas ACS in children usually presents at admission as a mild disease simulating community-acquired or viral pneumonia, the onset of the syndrome in adults is usually 1–3 days after hospitalisation for a severe VOC or bone pain crisis. Additionally, ACS in adults often runs a rapidly progressive course which may culminate in respiratory failure and is thus associated with high mortality rates. These differences may be attributable to the high prevalence of fat embolisation in adults and community-acquired or viral pneumonia in children.[8]

PATHOGENESIS OF ACUTE CHEST SYNDROME

An overview of the major pathogenic pathways involved in SCD is important in understanding the pathogenesis of ACS. Sickled red blood cells that arise from hypoxia-induced formation of sickle haemoglobin (HbS) polymers follow either the haemolytic or vaso-occlusive pathways. In the former, haemolysis releases free circulating haem which contributes to systemic endothelial dysfunction (haemolysis-related vasculopathy). On the other hand, sickled red blood cells can sequentially lead to vascular stasis, vaso-occlusion, and infarction (viscosity-vaso-occlusive phenomenon). The subsequent ischaemia-reperfusion results in systemic inflammation which also contributes to systemic endothelial dysfunction, characterised by increased adhesion molecules, reduced NO bioavailability, and increased vascular permeability. The systemic endothelial dysfunction is followed by increased red-cell and white-cell adhesion to vascular endothelium in target organs. For instance, two different phenotypes on pathogenesis have thus been proposed for sickle-cell nephropathy, namely 'haemolysis-endothelial dysfunction' phenotype and 'viscosity-vaso-occlusive' phenotype.[29] These phenotype models also equally explain some of the pathogenic pathways involved in SCD-related pulmonary complications. ACS, as an example of acute pulmonary injury, is initiated by multiple causative factors. The pathogenesis of the syndrome revolves around these major factors comprising pulmonary infections, pulmonary infarction, and pulmonary fat embolism (Figure 1).

Specific bacterial and viral pathogens have been identified as common infectious agents.[30] These include atypical bacteria such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and respiratory syncytial and influenza viruses. The infectious agents are believed to trigger ACS through excessive inflammatory response in the lungs, which arises from the systemic endothelial dysfunction.[10,31] Secondly, another pathway leading to ACS involves increased endothelial adhesion by sickled red cells and subsequent vaso-occlusion and pulmonary infarction: the viscosity-vaso-occlusion phenotype. The pulmonary infarction results in ventilation-perfusion (VQ) mismatch and worsening of hypoxaemia. Finally, pulmonary fat embolism triggers episodes of ACS through the pathogenic trajectory in which, at the onset, the VOC leads to bone marrow infarction and necrosis. This complication then deposits fat droplets into the circulation. Fat embolisation into the pulmonary vasculature initiates a metabolic cascade in which activated secretory phospholipase A2 cleaves fatty acids from phospholipids, releasing arachidonic acid. Subsequently, arachidonic acid is metabolised into eicosanoids which consist of the inflammatory

mediators leukotrienes and prostaglandins; these molecules mediate alveolar inflammation and injury to the pulmonary vascular endothelium.[32] Similarly, haem released from haemolysis contributes to the pulmonary vasculopathy (haemolysis-endothelial dysfunction phenotype), and initiates the development of ACS. In summary, ACS is characterised by a vicious cycle of pulmonary infarction, inflammation, and lung collapse, which is associated with VQ mismatch and hypoxaemia.[33] Furthermore, a study suggests that episodes of ACS render children with SCD more susceptible to increased lower airway obstruction.[34] This finding lends credence to the high case fatality rates associated with ACS in these children.

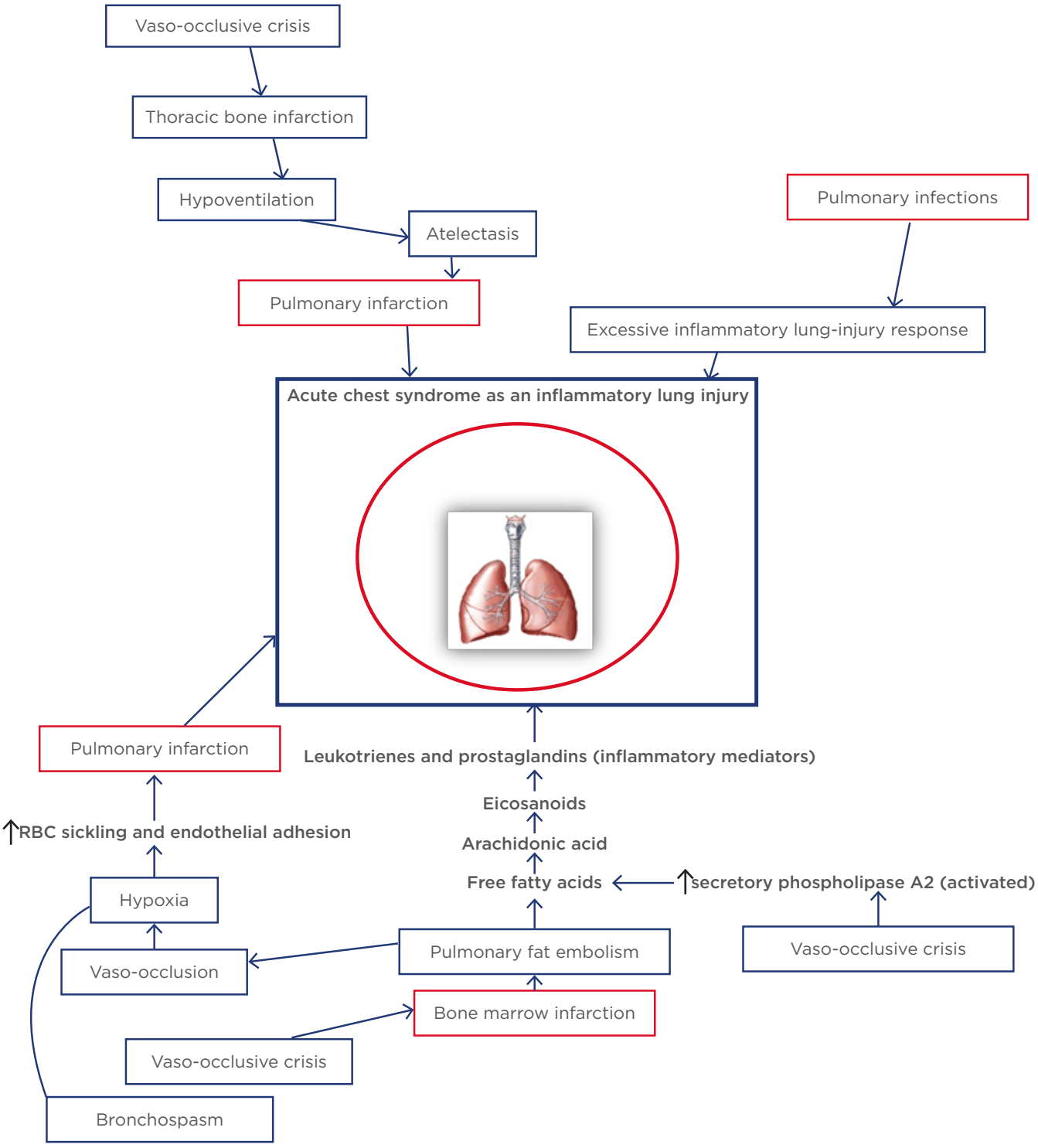

Figure 1: Schematic diagram of the pathogenesis of acute chest syndrome.

RBC: Red blood cell.

TREATMENT MODALITIES FOR ACUTE CHEST SYNDROME

ACS is best managed in hospital settings because intensive care of the patient may be required. The bedrock of treatment is supportive care. Recently, an expert-panel report from the National Heart, Lung, and Blood Institute (NHLBI) released evidence-based guidelines which have led to the effective treatment of SCD-related complications, including ACS.[35] Although the management of ACS is considered complex, the primary treatment modalities comprise supportive care, transfusion therapy, and pharmacotherapy (Table 1).

Table 1: A summary of treatment modalities in acute chest syndrome.

Treatment modalities	Treatment goals
Supportive care	
• Oxygenation supplementation	• To maintain SaO_2 level ≥94%
• Incentive spirometry or chest physiotherapy (by PEP)	• To prevent atelectasis
• Mechanical or noninvasive ventilation (e.g., CPAP)	• To support the work of breathing in the event of respiratory failure
• Cautious hydration with intravenous hypotonic fluids	• To prevent dehydration as well as reduce blood viscosity
Transfusion therapy/transplantation	
• Simple packed red cell transfusion	• To reduce the relative percentage of sickle red cells; minimise vaso-occlusion, bone pain, and hypoventilation
• Red cell exchange transfusion§	
• Chronic transfusion (hypertransfusion)	• To prevent recurrence of ACS by maintaining low levels of sickle red cells
• Haematopoietic stem cell transplantation (allogeneic)*	• To prevent recurrence of ACS that is refractory to treatment
Pharmacotherapy	
- Therapeutic measures	
• Analgesics: NSAID, opioids (using PCA)	• To reduce bone pain and thus circumvent hypoventilation
• Antibiotics/antivirals	• To eliminate the infective microbes responsible for the excessive inflammatory lung-injury response
• Corticosteroids†	• To suppress lung inflammation
• Bronchodilators‡	• To ameliorate bronchospasm-induced wheezing
• Inhaled nitric oxide‡	• To reduce bone pain crisis
• Anticoagulants (e.g., tinzaparin: a low molecular weight heparin)‡	• To reduce vaso-occlusion and SCD severity
Preventive measures	
• Hydroxyurea (hydroxycarbamide)	• To increase fetal haemoglobin levels and prevent recurrence of ACS

ACS: acute chest syndrome; CPAP: continuous positive airway pressure; NSAID: non-steroidal anti-inflammatory drugs; PCA: patient-controlled analgesia; PEP: positive expiratory pressure; SaO2: arterial oxygen saturation; SCD: sickle cell disease.

†: use still controversial; ‡: further research needed to validate its use; *: proven effective in children (modality of SCD cure); §: automated red cell exchange using a machine (erythrocytapheresis) or by manual red cell exchange (phlebotomy and transfusion).

The essential components of supportive care consist of sufficient oxygenation and ventilation, prevention of lung collapse, and cautious hydration. The goal of supplemental oxygen is to maintain the arterial oxygen saturation (SaO_2) level at ≥94%. Thus, ACS patients with SaO_2 values <94% or fast breathing require prompt oxygenation. When compared to pulse oximetry, which determines peripheral oxygen saturation level, arterial blood gas determination of SaO_2 is an ideal and accurate technique for measuring hypoxaemia in SCD patients who have abnormal haemoglobin-dissociation curves.[36] This pathophysiologic characteristic poses a challenge in resource-limited settings where pulse oximetry is more likely to be the available and accessible tool to measure hypoxaemia. Furthermore, inadequate monitoring and correction of hypoxaemia will worsen the VQ mismatch and contribute to fatal outcomes. Occasionally, SCD patients with chronic hypoxaemia may preclude the adoption of 94% as the limiting SaO_2 value for oxygenation. In this instance, a much lower value deviating from the baseline steady-state SaO_2 may be used to determine therapeutic intervention.

Signs of respiratory failure such as deteriorating hypoxaemia, severe breathing difficulty, and respiratory acidosis, may warrant supporting the work of breathing using noninvasive or mechanical ventilation. In fact, children with respiratory failure or disease progression who require ventilation support are best monitored and treated in paediatric intensive care units.[2] Despite the previous report indicating scant evidence for the use of noninvasive ventilation (continuous positive airway pressure) in children with ACS,[37] a more recent study suggests that its early use with blood transfusion was well tolerated in affected children.[38] Nevertheless, the role of noninvasive ventilation in changing patient outcomes has also been investigated in adult patients with ACS. A prospective randomised study reported improvements in respiratory outcome measures such as respiratory rate and gas exchange in these adult patients, albeit transient, but failed to document any change in blood transfusion rates: the intervention being associated with patient discomfort.[39]

A related component of supportive care is the prevention of lung collapse, which helps to circumvent the occurrence of hypoxaemia and VQ mismatch. Recommended methods of preventing lung collapse include incentive spirometry in wakeful state[40] and chest physiotherapy by positive expiratory pressure for younger patients who are either not able to comply with incentive spirometry or are breathing-constrained by thoracic wall pain.[41] In fact, both incentive spirometry and positive expiratory pressure resulted in the same outcome of preventing progression to ACS in a study of hospitalised children with SCD presenting with chest pain.[42] Furthermore, other authors have reported that incentive spirometry for children with SCD admitted without respiratory symptoms led to a decline in the incidence of ACS, especially for the cohort presenting with back pain.[40]

Hydration is also an essential aspect of supportive care. However, intravenous hypotonic fluids should be administered with caution because patients with ACS are susceptible to developing pulmonary oedema.[43] To circumvent pulmonary oedema, it is vital to deliver 75% of daily maintenance fluid and avoid intravenous bolus fluid.[9]

Concerning transfusion therapy, both simple and exchange blood transfusions are recommended in the treatment of ACS. Although an updated Cochrane review did not find enough evidence from randomised control trials to either support or refute the use of blood transfusion, the authors suggested that transfusion therapy should be individualised and experience driven.[44] According to the NHLBI expert-panel report, simple packed red blood cell transfusion (10 mL/kg) in ACS should aim to raise the haemoglobin level to 9–11 g/dL, or decrease the HbS level to >30%.[35] Compared to exchange blood transfusions, simple blood transfusions may not be able to reduce the HbS to this recommended target level without causing volume overload. Exchange blood transfusion therapy will perhaps be more beneficial in achieving this treatment goal because it increases the percentage of normal haemoglobin. At the same time, it reduces the percentage of HbS and the risk of iron overload from increased volume of transfused blood, and maintains the haematocrit levels at safe limits. It is therefore mostly used in patients who are not very anaemic.

Nevertheless, automated red cell exchange (RCE) transfusion or erythrocytapheresis is the recommended transfusion therapy for SCD patients who have severe and worsening ACS.[45] Similar to the effect of simple red cell transfusion, a double-volume RCE transfusion reduces the percentage of HbS to <20% and augments vascular perfusion.[46] This finding confirms a previous observation that both forms of blood transfusion result in the same outcomes in patients with ACS.[22] More importantly, double-volume RCE transfusion effectively, but transiently, reduces plasma inflammatory mediators such as white blood cells, absolute neutrophil count, platelets, and soluble VCAM-1 level.[47] This action may help mitigate the pulmonary vaso-occlusive sequelae that characterise ACS. Despite these advantages, the superiority of RCE transfusion over simple red cell transfusion has not been well established in clinical trials; an area that requires further investigation with appropriate study designs. Chronic blood transfusion, or hypertransfusion, is another type of transfusion therapy which is indicated in SCD patients with frequent ACS because it helps to prevent ACS recurrence by maintaining low levels of sickled red cells. However, it is potentially associated with the complication of iron overload. Finally, haematopoietic stem cell transplantation is advocated for SCD patients with eligibility criteria that consist of age <17 years, availability of HLA-matched sibling donor, and several complications which include severe and recurrent ACS.[48] Haematopoietic stem cell transplantation can prevent the recurrence of ACS, especially in cases refractory to pharmacologic agents such as hydroxyurea.

ACS is amenable to treatment with various pharmacologic agents. Firstly, antibiotics occupy a prominent place, given the role of pulmonary infections in its aetiopathogenesis. Empirical antibiotic therapy is based on the already documented epidemiologic patterns.[30] Thus, a regimen that comprises macrolides and third-generation cephalosporin with or without vancomycin is recommended as an ideal antibiotic therapy. However, with overuse of antibiotics and the consequent emergence of multidrug resistant bacteria, it may eventually become auspicious to institute routine blood cultures of pathogens to establish antibiotic-sensitivity patterns. A 10-day course of antibiotic therapy is considered optimal, although there are currently no guidelines on the maximum duration of therapy.[2] Administering antiviral agents has also been strongly advocated when seasonal influenza is under consideration, given the fact that children with ACS are more likely to have a viral aetiology than a bacterial aetiology.

Secondly, analgesic therapy is relevant in ACS patients because chest wall pain may be associated with thoracic splinting and hypoventilation, and is directly related to the evolution of the syndrome.[49] The current practice on pain management involves the use of a 'step-ladder' approach in which mild-to-moderate pain is treated with nonsteroidal anti-inflammatory drugs, while severe pain is managed with opioid analgesics, especially morphine.[50] However, in using the latter, the risk-benefit consideration is essential as the aim of treatment remains to achieve analgesia to obviate hypoventilation, while simultaneously avoiding opioid-related respiratory depression.[2] Thus, to achieve proper dosing of opioids to minimise these side-effects, patient-controlled analgesia could be used.[51] Parenteral opioids are administered through a microprocessor-controlled infusion pump which is used by patients once pain sensation occurs. Patient-controlled analgesia works on the basic principle of the patient's ability to evaluate pain intensity in VOC as well as analgesic potency. At the onset of pain, the patient activates the dosing system and receives a doctor-programmed analgesic dose, which is followed by activation of a protection system to prevent overdose.

With comorbid asthma and bronchospasm as a contributory risk factor for ACS, treatment with inhaled bronchodilators is advised, more so with the high incidence of lower airway obstruction and wheezing reported in ACS patients.[34] However, the evidence for their efficacy remains weak according to a recent Cochrane review.[52] Another inhalational pharmacologic agent adjudged as a potential medication in VOC is NO. On the contrary, a randomised control trial in hospitalised SCD patients with VOC did not document any significant difference in the resolution of the crisis and the episodes of ACS between NO and placebo groups.[53] Regarding the use of corticosteroids in ACS, which still

remains controversial, reports show that when administered in a low dose the drug could improve disease morbidity[54] and reduce the need for blood transfusion.[55] Nevertheless, documented adverse events such as haemorrhagic cerebrovascular accident[56] and recurrence of VOC after drug withdrawal[57,58] represent drawbacks to its regular use in ACS. Recently, anticoagulant therapy has also been considered as a modality in managing ACS. Specifically, tinzaparin (low-molecular-weight heparin) is known to reduce the duration and severity of pain associated with VOC by ameliorating vaso-occlusion.[59]

Hydroxyurea (hydroxycarbamide) is another pharmacologic agent that has been approved and used over the years as a disease modifier. It induces production of fetal haemoglobin with a concomitant decrease in the intracellular concentration of HbS, which affects the polymerisation of deoxygenated HbS. It also reduces the white cell count and the expression of cell adhesion molecules that contribute to vaso-occlusion and may also serve as a NO donor.[60,61] These actions underpin its role in the reduction of recurrence of ACS in children. Although there were initial concerns regarding its toxicity from long-term exposure, there is now reliable evidence supporting its use even in patients as young as 9 months of age, given its ability to reduce the frequency of VOC and ACS with minimal or absent side effects.[62]

CONCLUSION

ACS is associated with high mortality rates in children with SCD. Its aetiopathogenesis is predicated upon pulmonary infections, pulmonary infarction, and pulmonary fat embolism. The syndrome is characterised by a vicious cycle of pulmonary infarction, inflammation, and lung collapse, which is associated with VQ mismatch and hypoxaemia. Effective management thus requires a meticulous approach by the clinician to institute the primary treatment modalities comprising supportive intensive care, transfusion therapy, and appropriate pharmacotherapy. Whereas the efficacy of some of these modalities in attenuating or preventing ACS are well established, outcomes from instituting others are not yet convincing. For instance, more research is still needed to establish the superiority of exchange blood transfusion over simple blood transfusion, as well as the effectiveness of corticosteroids and bronchodilators in the management of the syndrome in children. Anticoagulant therapy using low-molecular-weight heparin (tinzaparin) also merits further attention and investigation.

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Therapeutic Options in Myelodysplastic Syndromes Following Hypomethylating Agent Failure

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Abstract

Hypomethylating agents (HMA) azacitidine and decitabine are standard of care for the treatment of myelodysplastic syndromes (MDS). Although HMA have revolutionised the treatment of MDS, only approximately half of patients respond to these agents with variable duration of effect, known as primary and secondary HMA failure, respectively. Therapeutic options following HMA failure remain limited; however, growing understanding of the pathogenesis underlying MDS has resulted in the development of multiple targeted therapies showing varying degrees of success in clinical trials. Drugs that target molecular alterations (such as abnormal histone regulation, *IDH* mutations, and spliceosome gene mutations), abnormal signalling pathways (such as the multikinase inhibitor rigosertib), cellular apoptosis (such as the Bcl2 inhibitor venetoclax), and immune checkpoint inhibition are under development. Agents recently approved for use in higher-risk acute myeloid leukaemia, such as FLT3-inhibitors and CPX-351, are also being studied in MDS. Several more agents, including two first-in-class agents, a novel immune regulator targeting CD47, and pevonedistat, a NEDD8-activating enzyme inhibitor, are under investigation. In the absence of established therapeutic approaches following HMA failure, decisions in therapy should be based on the type of HMA resistance as well as the patient's clinical and molecular characteristics. As targeted therapies continue to be developed, a comprehensive re-evaluation of the patient including the mutational profile at the time of HMA failure may reveal new treatment options. Here, emerging therapeutic approaches to HMA failure in MDS are reviewed.

INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of disorders defined by ineffective haematopoiesis and clonal instability with risk of transformation to acute myeloid leukaemia (AML).[1] Goals of therapy are to reduce the symptom burden from cytopenias and decrease the risk of progression of disease. Only three drugs have been approved by the U.S. Food and Drug Administration (FDA) for use in MDS: lenalidomide, an orally administered immunomodulatory drug; and two parenterally administered nucleoside analogue hypomethylating agents (HMA), azacitidine

and decitabine. HMA have been the standard of care for patients with MDS for over a decade.[2,3] Azacitidine, first approved in 2004, received expanded approval in 2008 for patients with higher-risk MDS based on the large, randomised Phase III AZA-001 trial which showed a median overall survival (OS) of 24.5 months compared to 15.0 months in patients receiving supportive care.[2] Decitabine was approved in 2006 based on Phase III study results showing an overall response rate (ORR) of 17% compared to 0% of patients receiving supportive care.[3] However, only azacitidine has been shown to prolong survival in MDS. While the European Medicines Agency (EMA) has approved HMA for International Prognostic Scoring System (IPSS; Table 1)[4] intermediate and higher-risk MDS, some countries such as the USA also utilise these agents in lower-risk patients. A revised IPSS (IPSS-R; Table 2) was developed in 2012; however, established therapies were approved using the original IPSS system.[5] Recent data suggests a benefit to early intervention in lower-risk patients.[6] Although HMA prolong survival, the response is transient and as many as half of patients will not respond to these agents. Moreover, there are different types of HMA failure, including absence of response, or progression of disease or failure following an initial response, termed primary and secondary failure, respectively. Regardless of the nature of failure, incapability to respond denotes a poor prognosis with models suggesting median OS of 4.5 months and 11.0 months in higher and lower-risk patients, respectively.[7] Therapeutic options following HMA failure remain limited with no standard of care approach. Fortunately, growing understanding of the pathogenesis of MDS and AML have led to the development of a variety of targeted therapies with varying degrees of success in clinical trials. Here, the mechanisms of HMA failure and novel therapeutic options in these patients are reviewed (Table 3).[8-55]

DEFINING HYPOMETHYLATING AGENT FAILURE

Patients should be treated with standard dose of HMA prior to assessing their response to therapy. There are currently two standard of care options: 1) decitabine 20 mg/m^2 per day for 5 days; or 2) azacitidine 75 mg/m^2 per day for 7 days, each at 4-week intervals for at least 6 cycles.[56] Interruption of therapy can lead to loss of response or disease progression while re-challenge may not be effective.[56] Moreover, recommendations from a consensus meeting of international experts recommended continuing HMA, if possible, until overt disease progression to minimise risk of relapse.[56] Routine follow-up during HMA therapy includes monitoring peripheral blood counts for cytopenias or blasts. Bone marrow evaluation is typically performed every 6 months, or earlier if progression of disease is suspected.[56] However, even when HMA therapy is optimised, failure may occur in different settings.

Primary response failure, or resistance, to HMA is defined by either the absence of response after at least four to six cycles of therapy demonstrated by stable disease without any of the following: complete remission (CR), marrow CR (mCR), partial remission (PR), or haematologic improvement (HI) based on the International Working Group (IWG) criteria,[57] progression of disease to higher-risk MDS category or to AML, or the discontinuation of therapy as a result of side effects such as hypoplastic marrow or pancytopenia.[58] Secondary response failure, or resistance, is defined by a loss of response or disease progression following an initial response (CR, mCR, PR, HI) to treatment.

The 'post-HMA model' was recently developed for prognostication following HMA failure. It incorporates the patient's Eastern Cooperative Oncology Group (ECOG) performance status, age at diagnosis, presence of complex cytogenetics, marrow blast percentage >20, red cell transfusion dependency, and platelet count <30,000.[59] Patients in this model are stratified into lower-risk or higher-risk with a median OS of 11.0 and 4.5 months, respectively.[59] Models like these could assist in clinical trial selection following HMA failure.[59] Although certain clinical and genetic features may predict favourable responses to HMA,[60] mechanisms of HMA resistance remain unclear.[61] Moreover, while MDS is characterised by DNA and gene-specific hypermethylation, the degree of demethylation following HMA administration is not associated with haematologic response.[62,63] Research efforts have focussed on identifying modifications in the cellular transport and metabolism of HMA as well as identifying biomarkers associated with HMA response.[64]

Table 1: International Prognostic Scoring System (IPSS).

		IPSS score				
		0.0	0.5	1.0	1.5	2.0
Prognostic variable	**Blasts (%)**	<5	5–10		11–20	21–30
	Cytogenetics±	Good	Intermediate	Poor		
	Cytopenias¥	0 or 1	2 or 3			

±Cytogenetics: good: normal, -Y only, *del(5q)* only, *del(20q)* only; Intermediate: abnormalities other than good or poor; Poor: complex >3 abnormalities, chromosome 7 abnormalities.

¥Cytopenias: haemoglobin <10 g/dL; absolute neutrophil count <1,500 cells/µL; platelet count <100,000 /µL.

IPSS risk score interpretation:
0.0 = Low risk
0.5–1.0 = Intermediate-1 risk
1.0–1.5 = Intermediate-2 risk
≥2.5 = High risk

Adapted from Greenberg et al.[4]

Table 2: Revised International Prognostic Scoring System (IPSS-R).

		IPSS-R score						
		0.0	0.5	1.0	1.5	2.0	3.0	4.0
Prognostic variable	**Blasts (%)**	≤2		>2 to <5		5 to 10	>10	
	Cytogenetics*	Very good		Good		Intermediate	Poor	Very poor
	Haemoglobin (g/L)	≥10		8 to >10	<8			
	Platelets, x10³µL	≥100	50 to <100	<50				
	Absolute neutrophil count, x10³/µL	≥0.8	<0.8					

*Cytogenetics: very good: *del(11q)* or -Y; Good: normal, *del(20q), del(5q), del(12p)*, or double including *del(5q)*; Intermediate: +8, *del(7q), i(17q)*, +19, or any other single or double independent clone; Poor: -7, *inv(3)/t(3q)/del(3q)*, double including *-7/del(7q)*, or complex (3 abnormalities); very poor: complex >3 abnormalities.

IPSS-R risk score interpretation:
≤1.5 = Very low risk
>1.5 to 3.0 = Low risk
>3.0 to 4.5 = Intermediate risk
>4.5 to 6.0 = High risk
>6.0 = Very high risk

Adapted from Greenberg et al.[5]

Table 3: Agents under active investigation in patients with myelodysplastic syndromes (MDS).

Mechanism	Agent	NCT identifier	Phase	Relevant study population	Status
Epigenetic regulators					
Hypomethylating agents	Guadecitabine	NCT02935361[8]	II	Int-1 or HR-MDS + HMAf	Recruiting
		NCT02131597[9]	II	HR-MDS	Active, not recruiting
		NCT02907359[10]	III	MDS + HMAf	
Histone deacetylase inhibitors	Vorinostat, mocetinostat, panobinostat, etc.	No active studies in this population			
Mutant IDH1/2 inhibitors	Enasidenib	NCT03383575[11]	II	*mIDH2* MDS +/- HMAf	Recruiting
		NCT03744390[12]	II		
	Ivosidenib	NCT02074839[13]	I	*mIDH1* r/r MDS	
		NCT03471260[14]	Ib/II	*mIDH1* MDS	
		NCT03503409[15]	II	*mIDH1*: HR-MDS, treatment-naïve MDS, ESA-resistant LR-MDS	
LSD1 inhibitors	Tranylcypromine	NCT02273102[16]	I	r/r MDS	Active, not recruiting
		NCT02717884[17]	II	Int-/HR-MDS + HMAf	Recruiting
	GSK2879552	No active studies			
Signal transduction regulators					
TGF-β signalling modulators	Galunisertib	No active studies in this population			
	Sotatercept				
	Luspatercept	NCT02631070[18]	II	Very low, low, or int-risk MDS refractory to ESA	Active, not recruiting
		NCT03682536[19]	III	Very low, low, or int-risk MDS in ESA-naïve	Recruiting
TLR inhibitors	Tomaralimab (OPN-305)	No active studies in this population			
Multi-kinase inhibitors	Rigosertib	NCT01926587[20]	I/II	Int-1, Int-2 (Int-2), or HR-MDS	Active, not recruiting
		NCT01904682[21]	II	LR or Int-1 risk-MDS	
		NCT01928537[22]	III	MDS + excess blasts + HMAf	
		NCT01241500[23]	III		
		NCT02562443[24]	III	Very high-risk MDS + HMAf	Recruiting
FLT-3 inhibitors	Midostaurin	NCT00819546[25]	I	r/r MDS and AML	Active, not recruiting
	Gilteritinib	No active studies in this population			
	Sorafenib	NCT02728050[26]	II	HR-MDS	Recruiting
Immunotherapy					
PD-1 inhibitors	Nivolumab	NCT02530463[27]	II	MDS +/- HMAf	Recruiting
		NCT02464657[28]	II		
		NCT03417154[29]	II		
	Durvalumab	NCT02775903[30]	II	Treatment-naïve, HR-MDS	Active, not recruiting
		NCT02281084[31]	II	MDS + HMAf	
	Pembrolizumab	NCT02936752[32]	I	MDS +/- HMAf	Recruiting
		NCT03094637[33]	II	Int-1 or HR-MDS +/- HMAf	
	Atezolizumab	NCT02935361,[8] see guadecitabine			
CTLA-4 inhibitors	Ipilimumab	NCT02530463,[27] see nivolumab			
		NCT02890329[34]	I	MDS +/- HMAf	Recruiting

Table 3 continued.

Mechanism	Agent	NCT identifier	Phase	Relevant study population	Status
Anti-CD47 antibody	Hu5F9-G4	NCT03248479[35]	I	r/r and treatment-naïve MDS	Recruiting
Bispecific T cell engaging antibodies	MCLA-117	No active studies in this population			
	AMG330				
	AMV564	NCT03516591[36]	I	Int-2 or HR-MDS with HMAf or standard AML CTX	Active, not recruiting
Cell death regulators					
Bcl-2 inhibitors	Venetoclax	NCT02966782[37]	I	HR-MDS + HMAf	Active, not recruiting
		NCT04017546[38]	I	MDS with ≥10% blasts	Recruiting
		NCT02942290[39]	I	Treatment-naïve HR-MDS	
		NCT03113643[40]	I	HR-MDS	
		NCT04160052[41]	I/II	HR-MDS +/- HMAf	
		NCT03404193[42]	II	HR-MDS + HMAf	
		NCT02115295[43]	II	MDS with ≥10% blasts	
NEDD 8 activating enzyme	Pevonedistat	NCT03772925[44]	I	HR-MDS + HMAf	Recruiting
		NCT03813147[45]	I	HR-MDS	
		NCT03459859[46]	I	MDS +/- HMAf	
		NCT03814005[47]	I	HR-MDS + HMAf	
		NCT03238248[48]	II	MDS+ HMAf	
		NCT02610777[49]	II	HR-MDS	Active, not recruiting
		NCT03268954[50]	III	HR-MDS	Recruiting
Other agents					
RNA splicing modulators	H3B-8800	NCT02841540[51]	I	HR-MDS + HMAf, LR-MDS refractory to ESA	Active, not recruiting
Cytotoxic agents	CPX-351	NCT02019069[52]	I	HR-MDS + HMAf	Recruiting
		NCT03896269[53]	I		
		NCT03957876[54]	II	MDS + HMAf	
		NCT03672539[55]	II	HR-MDS + HMAf	

CTX: chemotherapy; ESA: erythropoiesis-stimulating agents; HMAf: hypomethylating agent failure; HR: high risk; Int: intermediate; LR: low risk; MDS: myelodysplastic syndromes; *mIDH*: mutant *IDH*; r/r: relapsed/refractory; TLR: toll-like receptor.

CURRENT THERAPEUTIC OPTIONS FOLLOWING HYPOMETHYLATING AGENT FAILURE

Clinical trials are recommended for patients who fail HMA; however, if not accessible, chemotherapy and haematopoietic stem cell transplant (HSCT) may be used in the appropriate patient.

Cytotoxic Agents and Combinations

Standard AML-based chemotherapy, classically cytosine arabinoside plus anthracycline (7+3), may be used in patients with higher-risk MDS. However, high-dose chemotherapy is associated with prolonged cytopenias with serious infectious complications, and therefore is better tolerated by younger patients with more favourable cytogenetic profiles.[2,64] In a larger cohort of 307 patients, of whom 70% were lower risk, comparison of 3 induction regimens

(7+3, intermediate versus high dose cytosine arabinoside, or nucleoside analogues) showed similar median OS of 10.8 months, with ORR of 41%, 64%, and 34%, respectively.[65] Low-dose chemotherapy (including low-dose cytarabine, hydroxyurea, mercaptopurine, and low-dose melphalan) has not been shown to be superior to best supportive care (BSC), with median OS of 7.3 months.[66] However, in a study of predominantly elderly patients with higher-risk MDS after HMA failure, combinations of low-dose cytarabine and clofarabine resulted in a median OS of 10.0 months with 44% ORR.[67] Of responders, 30% underwent allogeneic HSCT and 56% achieved long-term remission.

A liposomal formulation of cytarabine and daunorubicin, known as CPX-351, was approved in August 2017 for therapy-related AML and AML with myelodysplasia-related changes.[68] Compared to standard 7+3, CPX-351 demonstrated improved OS (hazard ratio [HR]: 0.69; 95% confidence interval [CI]: 0.52–0.90; p=0.005), with a median OS of 9.6 months compared to 5.9 months.[68] Given the success of CPX-351, particularly in AML with myelodysplasia-related changes, researchers are investigating the effects of CPX-351 in elderly patients with MDS and AML following HMA failure in a Phase II clinical trial.[52]

Haematopoietic Stem Cell Transplant

Allogeneic HSCT is the only potentially curative option for patients with higher-risk MDS. However, given its significant toxicity and mortality, use is limited to younger patients or older patients who have a good performance status and donor. Moreover, studies suggest that patients with MDS who failed to respond to HMA have a higher risk of post-HSCT relapse than patients with positive response to HMA.[69] In 125 patients treated with HMA, relapse-free survival at 3 years was 23.8% in patients with primary HMA failure and 42.0% among patients who previously responded to HMA.[69]

EMERGING THERAPIES FOLLOWING HYPOMETHYLATING AGENT FAILURE

Novel Approaches to Hypomethylating Agent Therapy

Despite limited data, sequential use of the alternative HMA as a means of overcoming resistance is not uncommon practice. A few small, mostly retrospective studies have demonstrated between a 19% and 28% response rate to decitabine following azacitidine treatment failure[70,71] and 40% to azacitidine following decitabine treatment failure.[71]

Empiric addition of other agents to HMA in first-line therapy have been studied, including combinations with lenalidomide and vorinostat,[72] but have failed to show clinical benefit. Recently, a Phase II study employing a 'pick a winner' approach, investigated several combination therapies in patients with higher-risk MDS with the aim of launching more definitive investigations if a successful combination was found.[73] Patients were randomly assigned to treatment with azacitidine alone versus azacitidine plus lenalidomide, azacitidine plus valproic acid, or azacitidine plus idarubicin.[73] None of these combinations were found to be superior to azacitidine alone.[73] Ongoing combination therapies are being evaluated, including a Phase Ib trial investigating azacitidine with venetoclax[39] and a Phase III trial of azacitidine with pevonedistat.[50] Novel HMA with oral formulations, longer half-lives, and reduced toxicity are also under development.

Oral Hypomethylating Agents

Oral azacitidine and decitabine are currently undergoing evaluation in clinical trials. Compared to traditional parenteral formulations, oral formulations allow for delivery of the drug over a longer schedule and provide convenient dosing schedules for patients. Results from Phase I trials in patients with MDS and chronic myelomonocytic leukaemia (CMML) showed treatment with empiric oral azacitidine yielded an ORR of 73% compared to 35% in patients who were previously treated with traditional injectable formulations.[74] Findings from a Phase I study of oral azacitidine in lower-risk patients from this group demonstrated a benefit to extended

dosing schedules in which azacitidine given as 300 mg once daily for 14 versus 21 day dosing schedule resulted in an ORR of 36% and 41%, respectively.[75] A Phase III trial from this group is ongoing.[76]

Cedazuridine, a novel cytidine deaminase inhibitor called ASTX727, increases the bioavailability of oral decitabine by inhibiting its degradation in the gastrointestinal tract and liver.[77] Phase I studies of oral decitabine in combination with cedazuridine demonstrated similar clinical and biological responses when compared to intravenous decitabine.[77] Preliminary results from a Phase II study demonstrated clinical benefit in 62% of patients.[78] A Phase III study is ongoing.[77]

Guadecitabine

Guadecitabine is a novel, second-generation HMA resistant to deamination by cytidine deaminase and therefore exhibits longer half-life than decitabine. Two large, Phase II studies were conducted in patients with intermediate-2 or high-risk MDS and CMML either untreated[79] or following HMA failure.[80] In the first study, ORR was 61% with a median OS of 15.0 months at a median follow up of 15.0 months.[79] The second study, which included patients with HMA failure and treatment-naïve patients, achieved an ORR of 43% and 51%, respectively, with median OS of 12.0 and 23.1 months.[80] These results support guadecitabine in first-line therapy. A Phase III trial comparing guadecitabine to standard therapy in patients with MDS and CMML after HMA failure is underway.[10]

OTHER DRUGS TARGETING EPIGENETIC DYSREGULATION

Histone Deacetylase Inhibitors

One of the key mechanisms in the epigenetic regulation of gene expression is through histone acetylation and deacetylation. Histone deacetylation results in transcriptional deactivation and ultimately downregulation of gene expression. Transcriptional repression complexes, such as histone deacetylases (HDAC), may downregulate tumour suppressor genes.[81] Histone deacetylase inhibitors (HDACi) have been assessed for use in MDS alone or in combination with HMA. HDACi may also play a role in apoptosis and induce alterations in the NF-κB pathway.[81,82] Despite robust preclinical data and extensive studies in patients with MDS and AML, HDACi have not been shown to improve outcomes in combination therapy with HMA.[81,83-85]

Mutant *IDH1* and *IDH2* Inhibitors

Isocitrate dehydrogenases (IDH) are enzymes involved in diverse cellular processes, including histone demethylation and DNA regulation.[86] Mutations in *IDH1* or *IDH2* (*mIDH1/2*) result in DNA and histone hypermethylation impeding haematopoietic progenitor cell differentiation and promoting leukaemogenesis.[86] Together, *mIDH1/2* are among the most common mutations in myeloid malignancies, occurring in approximately 20% of patients with AML and 5% with MDS.[87] Several *mIDH1/2* inhibitors are under development as monotherapy or in combination with HMA or chemotherapy. These include two oral agents recently approved by the FDA for use in AML: enasidenib, a *mIDH2* inhibitor approved for relapsed/refractory AML with *IDH2* mutation; and ivosidenib, a mIDH1 inhibitor approved for AML with *IDH1* mutation.

Enasidenib received FDA approval in August 2017 following a Phase I/II study in relapsed/refractory AML achieving ORR 40.3% with CR 19.3%.[88] A significant survival benefit was seen in patients achieving CR with median OS of 19.7 months versus 9.3 months in relapsed/refractory pateints.[88] Enasidenib is now being evaluated in patients with MDS after HMA failure, including in two Phase II studies.[11,12]

Similarly, ivosidenib received FDA approval in July 2018 for relapsed/refractory AML based on results from a Phase I study of 125 patients achieving ORR of 42.0% after 7 months, with CR 22.0% with median duration of 9.0 months. The median OS was 9 months after a median follow-up of 15.0 months.[89] Results from an expansion study of 12 patients showed ORR of 91.7%, with median duration 21.4 months and CR 41.7% (median duration not estimable).[90] Ivosidenib was recently approved as first-line therapy in AML in May 2019. Ivosidenib is being evaluated in a Phase II study in patients with MDS following HMA failure.[15]

Lysine Demethylase 1 Inhibitors

Lysine demethylase 1 (LSD1) regulates gene transcription through the removal of methyl groups from histones and is overexpressed in myeloid malignancies. LSD1 inhibitors (LSD1i) have been shown to promote the differentiation of blast cells in AML, particularly in patients with mutations in *KMT2A*.[91] A Phase I/II study of LSD1i GSK2879552 in MDS[92] and Phase I study in AML were both terminated early; however, tranylcypromine, another LSD1i, is currently being evaluated in Phase I/II studies with results pending.[16,17]

DRUGS TARGETING ABNORMAL SIGNAL TRANSDUCTION

TGF-β Receptor Signalling Modulators

Abnormal activation of the TGF-β receptor signalling pathways has been implicated in the pathogenesis of MDS representing a novel therapeutic target, while suppression of this pathway promotes *in vitro* haematopoiesis in MDS progenitor cells.[93]

Sotatercept, a selective activin receptor ligand that traps GDF11 to restore effective erythropoiesis, was studied in 74 patients, 48% of whom had HMA failure. 49% of patients achieved erythroid HI, as did 59% of patients with HMA failure and 47% with high transfusion burden.[94]

Galunisertib, a first-in-class oral inhibitor of the TGF-β receptor type 1 kinase (ALK5), was recently evaluated as monotherapy in 43 patients with very low, low, or intermediate-risk MDS.[95] Overall HI was 24.4% (10/41) and 31.1% in transfusion-dependent patients (9/28) with a median response duration of 3 months. Two patients were previously treated with HMA. Although these agents have yet to be tested in higher-risk patients, findings from these studies suggest treatment with sotatercept and possibly other TGF-β receptor signalling modulators may be an option in transfusion-dependent, lower-risk patients following HMA failure.

Toll-like Receptor Inhibitors

Toll-like receptors (TLR) play a key role in innate immune activation through activation of NF-κB. Overexpression of TLR2 on the MDS cell membrane, which is upregulated by HMA therapy and may be implicated in HMA failure, has been shown to inhibit haematopoietic differentiation in MDS.[96] Phase I/II studies using tomaralimab (OPN-305), a fully humanised IgG4-κ monoclonal antibody against TLR2, were conducted in patients with lower-risk MDS following HMA failure.[96] Preliminary data suggest an ORR of 50%, supporting a role for tomaralimab in the treatment of lower-risk patients with HMA failure.[96]

Rigosertib Alone and in Combination with Azacitidine

Rigosertib is a multi-kinase inhibitor of cellular signalling through the targeting of the Ras-binding domain of RAS, PI3K/AKT, and RAF/PLK, inducing mitotic arrest and apoptosis in neoplastic cells. Rigosertib is currently undergoing investigation as a single agent in certain subtypes of MDS, including patients with higher-risk MDS following HMA failure, as well as in combination with azacitidine. Although intravenous rigosertib was not shown to improve OS compared to BSC in higher risk patients with HMA failure in the Phase III ONTIME trial, a post hoc analysis of very high-risk patients showed median OS significantly improved to 7.6 months in the study group compared to 3.2 months in BSC.[97] Survival benefit was seen in patients with primary HMA failure, monosomy 7 or trisomy 8, and who were <75 years of age.[97] A second Phase III study is underway, which will further evaluate these patients with very high-risk disease.[24]

An oral formulation of rigosertib is also under investigation. Preclinical data demonstrated a synergistic effect with sequential dosing of rigosertib with azacitidine.[98] This combination was evaluated in a Phase I/II study of 74 patients with higher-risk MDS.[99] A dose of >840 mg per day resulted in ORR 90% and 54% in HMA naïve and HMA failure patients, respectively.[99] A Phase III study is anticipated.

FLT3 Inhibitors

The *FLT3* gene encodes a tyrosine kinase receptor expressed on haematopoietic progenitor cells, which promotes cellular proliferation and differentiation. *FLT3* is mutated in approximately 30% of patients with AML, conferring a poor prognosis with resistance to conventional chemotherapy regimens.[100] The FLT3 inhibitors midostaurin and gilteritinib have been approved by the FDA in *FLT3*-mutated AML, the former in combination with 7+3. Although *FLT3* mutations are seen in <1% of patients with newly diagnosed MDS, they are found in up to 5% of patients with MDS transformed to AML.[101] Phase I/II studies of the FLT3 inhibitors midostaurin and sorafenib, in combination with azacitidine, demonstrated efficacy with ORR of 26% and 46%, respectively. Although the majority of the study participants had AML, these agents show promise in *FLT3*-mutated MDS.

IMMUNOTHERAPY

Immune Checkpoint Inhibitors

Immune regulatory proteins PD-1/PD-L1 and CTLA-4 downregulate antitumour T-cell responses and promote tumourigenesis. These immune regulatory proteins were upregulated in MDS cells treated with HMA,[102] which may be linked to HMA failure. The PD-1 inhibitor nivolumab, as well as the CTLA-4 inhibitor ipilimumab, were evaluated in a Phase II study in combination with azacitidine in treatment-naïve MDS or as monotherapy in patients with HMA failure.[103] In treatment-naïve patients, ORR was 70% with nivolumab/azacitidine and 62% with ipilimumab/azacitidine. Median survival was not reached at a median follow up of 20.1 months in treatment-naïve patients treated with ipilimumab/azacitidine, surpassing the effect of azacitidine alone. Further investigations into these agents, including triple combinations,[27,104] are under investigation.

Anti-CD47 Antibodies

Hu5F9-G4 (5F9) is a first-in-class anti-CD47 antibody, which targets a key macrophage immune checkpoint resulting in AML cell phagocytosis.[105] Azacitidine enhances phagocytic elimination of AML cells when combined with 5F9.[106] A Phase I study of 5F9 alone or in combination with azacitidine in patients with relapsed/refractory AML and MDS among other cohorts is under investigation.[35]

Bispecific T-cell Engaging Antibodies

Bispecific T-cell engaging antibodies link T cells (via the CD3 receptor) with specific antigens on tumour cells to induce tumour cell apoptosis. An increasing number of tumour-specific antigens are under development, including CD33 and CLEC12A, which are frequently expressed on myeloid precursors in AML and MDS.[107,108] Two novel bispecific CD33/CD3 antibodies, AMG330 and AMV564, and a bispecific CLEC12A/CD3 antibody, MCLA-117, are being evaluated in Phase I studies in relapsed/refractory AML.[109-111] Preliminary results of AMV564 have been encouraging, with reduction in myeloblasts ranging from 13 to 38% in 6/9 evaluable patients.[112] AMV564 is also undergoing investigation in intermediate and high-risk MDS in a Phase I study.[36]

RNA splicing modulators

Dysregulated mRNA splicing has been implicated in tumourigenesis. Genes involved in spliceosome machinery, including *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*, are frequently mutated in patients with MDS representing novel therapeutic targets.[113] Based on positive results from preclinical trials, a Phase I study of a novel splicing modulator, H3-B8800, is under investigation in MDS patients with HMA failure, CMML, and AML.[51]

DRUGS TARGETING DEREGULATED CELL DEATH PATHWAYS

Bcl-2 Inhibitors

Bcl-2 is a mitochondrial protein that promotes cellular survival by inhibiting pro-apoptotic pathways. Overexpression of Bcl-2 has been reported in higher-risk MDS leading to resistance of apoptosis[114] and to azacitidine.[115] Venetoclax is an orally bioavailable potent inhibitor of Bcl-2. In November 2018, venetoclax was approved for older adults with newly diagnosed AML in combination with HMA or low-dose cytarabine who were otherwise not candidates for intensive induction therapy. Interim analysis of a Phase

II study[42] of venetoclax in combination with decitabine achieved a CR/CR with incomplete blood count recovery of 92% in older patients with newly diagnosed AML, 71% in secondary AML, and 44% in patients with relapsed/refractory AML.[116] Given its success in AML, venetoclax is also being investigated in MDS. A Phase Ib study will examine the effect of venetoclax alone or in combination with azacitidine in high-risk patients following HMA failure.[37]

Pevonedistat

Pevonedistat is a first-in-class inhibitor of the NEDD8 activating enzyme (NAE). NAE is an essential regulator of the degradation of proteins involved in cell cycle progression and cellular stress responses. Preclinical data demonstrated the effect of NAE inhibition in inducing AML cell death[117] as well as synergistic effects with azacitidine and decitabine.[118] A Phase I study of pevonedistat plus azacitidine in treatment-naïve, older patients with AML showed intention-to-treat ORR of 50%.[119] This combination is being evaluated in a Phase II study in patents with MDS after HMA failure.[48] The Phase III PANTHER trial is comparing pevonedistat plus azacitidine to azacitidine alone as first-line therapy in higher-risk MDS, CMML, and low-blast AML.[50]

CONCLUSION

It is critical to recognise HMA failure in MDS because these patients have poor outcomes. Although there are no standard therapeutic options following HMA failure, several emerging therapies with the goal of improving symptom burden and overall survival are showing promise in clinical studies. These include novel targeted therapies and immune therapies to genes commonly altered in MDS. Therefore, clinical trial enrolment is the preferred option after HMA failure. A comprehensive assessment of the patient's clinical, molecular, and cytogenetic profiles at the time of HMA failure will help guide therapy selection.

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Reed Sternberg-Like Cells in an Aggressive Lymphoma: Report of a Rare Case and Review of Literature

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Abstract

Peripheral T-cell lymphomas are aggressive lymphomas with a rapidly progressive clinical course and sinister prognosis even with the best available treatment modalities. Epstein–Barr virus-positive peripheral T-cell lymphoma is an unusual variant of the disease; it is extremely rare and associated with a fulminant course spanning weeks to months. Treatment protocols are different for this entity because of its rarity.

INTRODUCTION

The World Health Organization (WHO) has defined peripheral T-cell lymphomas (PTCL) as a heterogenous group that is diagnosed based on typical histological findings: medium-sized or large cells with irregular, pleomorphic nuclei; prominent nucleoli and many mitotic figures; an aberrant immunohistochemical profile with the loss of CD5 and CD7, along with a clonal T-cell receptor gene rearrangement. It is associated with poor prognosis with a rapidly progressive clinical course. Epstein–Barr virus (EBV)-positive (+) PTCL is an uncommon variant with worse prognosis than PTCL not otherwise specified.[1] In low-grade B-cell lymphomas, Reed Sternberg (RS)-like cells can be seen in follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma, and marginal zone lymphoma. Among the T-cell lymphomas, RS-like cells are seen in angioimmunoblastic lymphoma and peripheral T-cell lymphoma.[2,3]

CASE REPORT

A 27-year-old female presented with history of fever, weight loss, and persistent, enlarged neck swellings for 3 months. She had been diagnosed with HIV 4 years previously, and with tuberculous lymphadenitis 2 months prior to this study and started on antitubercular therapy (ATT); however, she stopped taking ATT after 1 month because of persistent vomiting and yellowish discolouration, potentially owing to ATT-induced hepatitis.

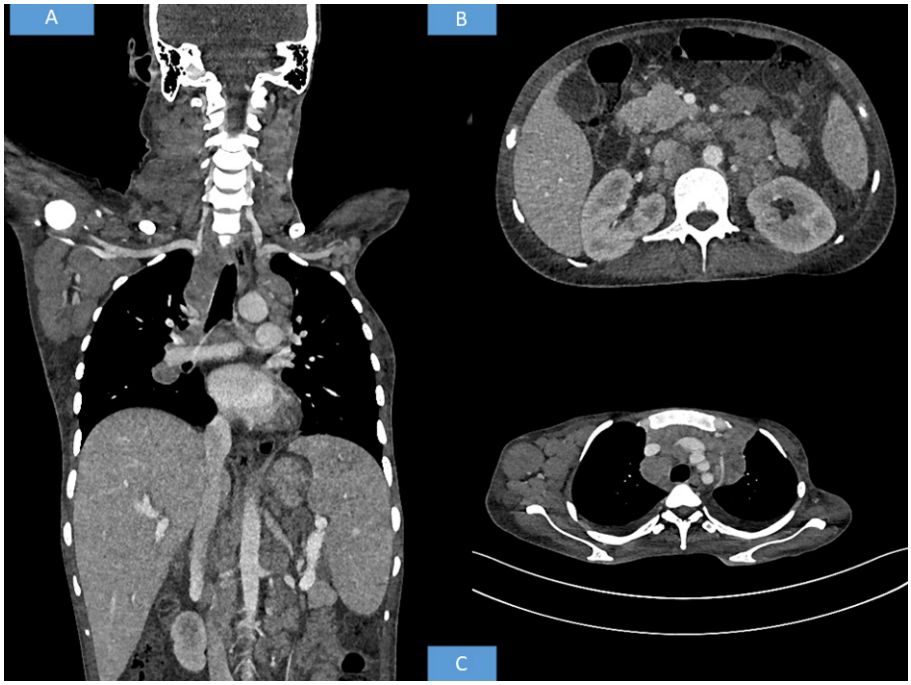

Figure 1: Radiological findings. (A) Whole body CT shows hepatosplenomegaly with axillary and cervical lymphadenopathy. **(B)** CT shows retroperitoneal enlarged lymph nodes. **(C)** CT shows axillary and mediastinal lymphadenopathy.

Clinical examination revealed multiple cervical and axillary lymphadenopathy ranging in size from 1.5 to 3.0 cm. The bilateral supraclavicular nodes were also enlarged. On abdominal examination, gross ascites with hepatosplenomegaly was noted. Haematological examination showed pancytopenia with a macrocytic blood picture. Ultrasonography of the abdomen was suggestive of ascites with hepatosplenomegaly and retroperitoneal lymphadenopathy.

Contrast-enhanced CT of the neck, thorax, abdomen, and pelvis was advised. The CT revealed multiple discrete homogenously enhancing enlarged lymph nodes seen in cervical stations with conglomeration in few areas involving right Level II, right Level III, right Level IV, right axillary, and bilateral supraclavicular nodes. Mediastinal lymph nodes were also enlarged with bilateral pleural effusion. The enlarged preaortic, para-aortic, aortocaval, retroperitoneal lymph nodes were seen encasing and lifting the abdominal aorta without any luminal narrowing. Along with gross ascites and hepatosplenomegaly, multiple hypodense splenic deposits were also noted. Based on the above radiological findings, the possibility of a lymphoma was suggested (Figure 1).

Ultrasonography-guided ascitic tap was performed and sent for cartridge based nucleic acid amplification testing which gave a negative result. A lymph node biopsy was done from right Level II lymph node and sent for histopathological examination and cartridge based nucleic acid amplification testing which, again, was negative. Haematoxylin-eosin stained sections revealed diffuse effacement of the nodal architecture by small to medium-sized lymphoid cells with round or convoluted nuclei and scant cytoplasm. Interspersed between these cells were large pleomorphic cells with scant to moderate amounts of eosinophilic to vacuolated cytoplasm, large oval to irregular nuclei, vesicular chromatin, and prominent nucleoli resembling the owl eye appearance of RS cells. Based on the histological findings, the possibility of lymphocyte-depleted Hodgkin's lymphoma and anaplastic large-cell lymphoma were considered and immunohistochemistry was ordered.

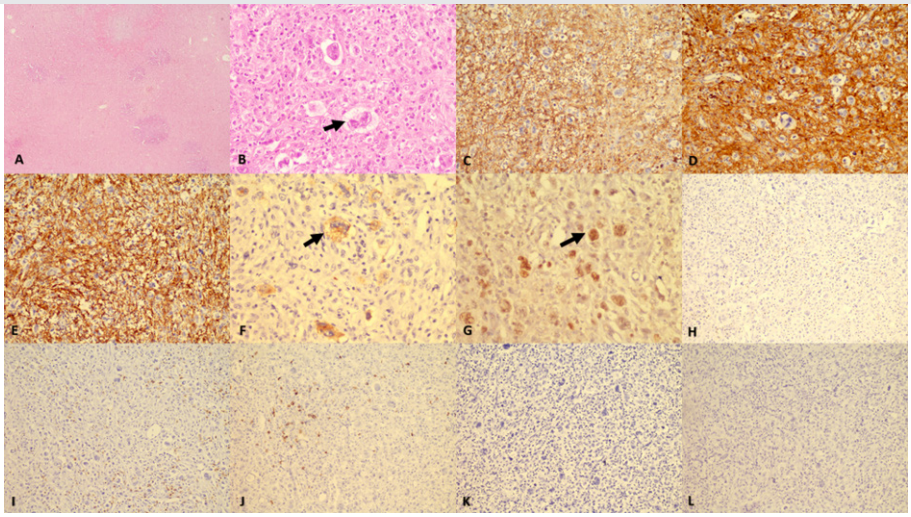

Figure 2: Histomorphology and immunohistochemistry findings. (A) Low magnification (40x haematoxylin and eosin [H&E]) shows complete effacement of lymph node architecture with diffuse infiltration by atypical cells. **(B)** Higher magnification (400x H&E) shows, aside from tumour population, numerous large cells (arrow) with prominent eosinophilic nucleoli resembling Reed Sternberg cells. In background, eosinophils are also seen. The tumour cells are diffusely stained with **(C)** LCA; **(D)** CD 4; and **(E)** CD68. The bizarre tumour cells show strong staining (arrow) with **(F)** EBV LMP-1; and **(G)** GATA-3. The tumour cells are negative for **(H)** CD3; **(I)** CD8; **(J)** CD20; **(K)** CD30; and **(L)** PAX-5.

Immunohistochemistry showed that the small to medium-sized cells were positive for CD4 and CD68 while the large pleomorphic cells were only positive for EBV-latent membrane protein 1 (LMP). The bizarre cells did not show positivity for any B-cell markers (CD20, PAX5, CD79a) or for CD15, CD30, Oct-2, and Bob-1, which are positive in RS cells. HMB-45, CD1a, langerin, anaplastic lymphoma kinase, EMA, vimentin, desmin, cytokeratin, and CD56 were also tested to exclude the possibility of malignant melanoma, anaplastic large cell lymphoma, high-grade carcinoma, and natural killer cell lymphoma, and all turned out to be negative. Two differentials were concluded: EBV+ PTCL and histiocytic sarcoma. GATA Binding Protein 3 (GATA3) was positive in the large cells therefore a final diagnosis of EBV+ PTCL was made (Figure 2).

The patient was initially advised treatment with cyclophosphamide and prednisolone for lymphoma rather than full CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) treatment which could not be administered because of deranged liver function. The patient was counselled regarding the decision and she agreed to the treatment; however, the patient was not reachable during the follow-up. Upon further contact it was revealed that the patient had died.

DISCUSSION

PTCL is a heterogeneous group that can have both nodal and extranodal presentation and does not fit the specifically defined entity of mature T-cell lymphoma. It usually presents in adults with a male:female ratio of 2:1. It is associated with rapid progression of clinical course.[1] EBV+ PTCL, a rare variant of PTCL, is more frequent in late adulthood and has a poorer prognosis. Patients are more likely to have B symptoms and hepatic involvement. A higher stage, usually Stage III/IV, is seen in EBV+ cases.[1]

Histological examinations of PTCL show a mixture of small to large atypical lymphoid cells with clear cytoplasm and irregular nuclei with conspicuous nucleoli; however, EBV+ PTCL show different histomorphological findings with centroblastic, anaplastic, or plasmacytoid appearance.[4] Immunohistochemical profiles of PTCL show double positive CD4+>CD8+ with aberrant phenotypes associated with loss of CD5 and CD7.[5] EBV+ PTCL are CD8+/CD4-, double

negative, and approximately 15% are CD4+/CD8-. Cytotoxic molecules such as TIA-1 and granzyme are expressed in nearly all cases unlike in PTCL not otherwise specified in which they may or may not be expressed.[5]

Immunopositivity for GATA3 has been associated with a poorer prognosis with increased infiltration with tumour-associated macrophages and higher CD68 positivity as in seen in this report.[6] The pathogenesis of EBV-associated PTCL was proposed by Cheng et al.[7] by two mechanisms. Firstly, EBV-associated proteins such as EBV nuclear antigen 1 or LMP transactivate certain important genes in lymphoma cells thus further stimulating the cytokine secreting T cells. Secondly, EBV may initiate an abnormal immunological reaction of host against the EBV+ T lymphoma cells.[7]

The aggressive course of PTCL has been attributed to a terminal haemophagocytic syndrome and rapid emergence of P-glycoprotein following a course of chemotherapy.[7] Two types of haemophagocytic syndrome have been reported. Jaffe et al.[8] described one in which a disease-free period was seen followed by development of terminal haemophagocytic syndrome. Bone marrow in the above subtype of syndrome showed many reactive histiocytes.[8] The second type of haemophagocytic syndrome was described by Falini et al.[9] in which there was simultaneous development of haemophagocytic syndrome with lymphoma followed by death after a few months. In contrast to the former, bone marrow, liver, and spleen in this syndrome showed reactive histiocytes along with lymphoma cells.[9] Song et al.[10] and Wang et al.[11] have also reported similar cases in which, alongside generalised lymphadenopathy, erythematous papules were also present. Both cases were aged between 40 and 49 years old and immunocompetent, while the patient in this case report was in their 20s and immunocompromised. The authors' case did not show any skin manifestations. Histomorphological findings in the above cases were similar to the case in the present report; however, on immunohistochemical analysis, the larger RS-like cells were positive for EBV-LMP and GATA3 and immunonegative for B-cell markers (CD20, CD79a). In contrast, their cases showed the large pleomorphic cells to be immunopositive for CD20, PAX-5, and CD30. They tested EBV positivity by EBV-encoded RNA *in situ* hybridisation while in this present case it was carried out using immunohistochemistry for EBV-LMP.

Goh et al.[12] reported a similar case of a patient in their 50s with presentation of isolated inguinal lymphadenopathy. Mori et al.[13] reported a case of a patient in their 70s presenting with gradual enlargement of submandibular nodes which was earlier misdiagnosed as classical Hodgkin lymphoma. The case was treated as Hodgkin lymphoma, which then recurred, only to be diagnosed as angioimmunoblastic lymphoma on a subsequent biopsy. Butler et al.[14] reported a similar case of a patient in their 50s who presented with pyrexia of unknown origin. It was earlier misdiagnosed as Hodgkin lymphoma; however, a subsequent excisional biopsy revealed PTCL with RS-like cells. Willemsen et al.[15] reported a case of angioimmunoblastic lymphoma with RS-like cells in a 76-year-old male patient who presented with chylothorax and chylous ascites; however, these RS-like cells were negative for EBV on EBV-encoded RNA *in situ* hybridisation.

CONCLUSION

The authors have reported a case of EBV+ PTCL in a 27-year-old patient. The importance of diagnosing this entity lies in the fact that it is a rapidly progressive lymphoma associated with very poor prognosis possibly owing to the association with terminal haemophagocytic syndrome and expression of P-glycoprotein. It is very important to differentiate a composite lymphoma from a T-cell lymphoma with reactive RS-like cells as treatment modalities are very different.

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Pernicious Anaemia: Mechanisms, Diagnosis, and Management

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Abstract

Pernicious anaemia (PA) is an autoimmune disease of multifactorial aetiology involving environmental and immunological factors. It is the most common cause of cobalamin deficiency anaemia worldwide. The disease is a macrocytic anaemia caused by a vitamin B12 deficiency, which, in turn, is the result of intrinsic factor deficiency, a protein that binds avidly to dietary vitamin B12 and promotes its transport to the terminal ileum for absorption. Despite the advances in understanding the pathogenesis and molecular biology, diagnosis of PA is still challenging for clinicians because of its complexity, diverse clinical presentations, and the limitations of the available diagnostic tools for the evaluation of cobalamin status and the presence of chronic autoimmune atrophic gastritis. Asymptomatic autoimmune gastritis, a chronic inflammatory disease of the gastric mucosa, precedes the onset of corpus atrophy by 10–20 years. Diagnostic dilemmas could occur when patients with PA present with spuriously normal or high cobalamin levels, normocytic or microcytic anaemia, nonanaemic macrocytosis, autoimmune haemolytic anaemia, pseudo-thrombotic microangiopathy, hyperhomocysteinemia-associated thromboembolism, pseudoleukemia, bone marrow failure, and neurologic manifestations without anaemia or macrocytosis. Other autoimmune disorders, especially thyroid disease, Type 1 diabetes mellitus, and vitiligo, are also commonly associated with PA. The present review focusses on novel aspects regarding the pathogenesis, clinical presentation, and the diagnostic approach of PA; the true usefulness of serum vitamin B12 levels; and the risk of adenocarcinoma and gastric carcinoids as well as their treatment and monitoring strategies.

INTRODUCTION

Pernicious anaemia (PA) (also known as Biermer's disease[1] and Addisonian anaemia[2]) is a macrocytic anaemia caused by a vitamin B12 (cobalamin) deficiency, which, in turn, is the result of intrinsic factor (IF) deficiency. IF is a glycoprotein that binds cobalamin and thereby enables its absorption at the terminal ileum.[3]

Whether the stomach pathogen *Helicobacter pylori* plays a causative role in PA is unclear. The deficiency of IF is a consequence of the presence of atrophic gastritis, which results in the destruction of the oxyntic mucosa and thus the loss of parietal cells, which normally produce hydrochloric acid as well as IF.[4] Acid loss leads to iron deficiency anaemia that precedes cobalamin-deficient PA by many years. PA is widespread across all continents and the prevalence of the disease ranges from 50 to 4,000 cases per 100,000 persons, depending on the diagnostic criteria.[5] However, PA prevalence is probably underestimated as a result of the complexity of the diagnosis. The incidence of PA increases with age and is rare in people <30 years of age. The mean age of patients with PA ranges from 59 to 62 years. PA is more common in people with African or European ancestry than in those with Asian ancestry. The highest prevalence is seen in northern Europeans, especially those in the UK and Scandinavian countries.[6,7] The symptomatology is dominated by a profound megaloblastic type anaemia and, in the most serious cases, by neurological alterations, which can precede the diagnosis of gastric atrophy by several decades. Furthermore, PA correlates with other autoimmune diseases (thyroiditis and Type 1 diabetes mellitus) as well as a genetic disease (genotypes *HLA-DRB1*03* and *DRB1*04*);[8,9] however, differential diagnosis may sometimes be challenging because of the limitations of currently available diagnostic tools.

PATHOPHYSIOLOGY

Physiological Roles of Vitamin B12

Vitamin B12, or cobalamin, is an hydrosoluble vitamin synthesised by micro-organisms and detected in trace amounts mostly in foods of animal origin. The normal daily cobalamin requirement for an adult corresponds to 1–2 nmol/L. The absorption and transport mecchanisms are dependent on three key proteins: haptocorrin (HC), IF, and transcobalamin (TC).

In the stomach, vitamin B12 released from food protein by peptic action is bound to HC and travels to the duodenum, where pancreatic proteases digest HC, releasing vitamin B12 to bind to IF. The IF–vitamin B12 complex binds to a specific receptor called cubilin in the distal ileum and is internalised. Eventually it is released by lysosomes, and transported into the blood. Both HC and TC bind circulating vitamin B12 and although the latter is the cellular delivery protein, it is transported together with TC I, II, or III to finally be stored in the hepatocyte.[4,5]

Cobalamin acts as a fundamental enzymatic cofactor in myelopoiesis (role in nucleotide synthesis) and myelination of the central and peripheral nervous system. The two enzymes through which vitamin B12 serve this function are methionine synthase and methylmalonyl-CoA mutase. Thus, cobalamin serves as a cofactor for methionine synthesis through the transfer of a methyl group to homocysteine which is an atherogenic and potential endothelial toxin. This conversion of homocysteine to methionine forms demethylated tetrahydrofolate which is required for DNA synthesis. Further metabolism of methionine to S-Adenosyl methionine is essential for myelin synthesis and the maintenance of neuronal integrity, as well as for neurotransmitter regulation. Thus, a lack of cobalamin leads to either the destruction of myelin sheaths or the incorporation of abnormal fatty acids into myelin sheaths, thereby leading to impaired neural function and/or transmissions which may be the underlying cause of the neurological symptoms seen in vitamin B12 deficiency.

The resulting anaemia may be macrocytic with bone marrow promegaloblastosis, reflecting ineffective erythropoiesis, or normocytic reflecting concomitant iron deficiency from achlorhydria.

Dyssynchrony between the maturation of the cytoplasm and that of the nuclei leads to macrocytosis, immature nuclei, and hypersegmentation in granulocytes in the peripheral blood. The ineffective erythropoiesis results in intramedullary haemolysis and the release of lactate dehydrogenase (LDH).[4,8] Inhibition of DNA synthesis as a result of vitamin B12 deficiency causes megaloblastic changes not only in bone marrow but also in other rapidly dividing cells, such as gastrointestinal epithelial tissue, explaining gastrointestinal disorders in patients with PA.

Physiopathology of Pernicious Anaemia

PA is a complex, autoimmune, multifactorial disease. The environment appears to play a crucial, independent role in the pathogenesis of PA. Even though PA associated with gastric atrophy is now considered an outcome of chronic *H. pylori* infection, the relationships between PA and *H. pylori* is still not clear with conflicting views.[10-14] *H. pylori* are ubiquitous organisms invading the gastric mucosa and are a global burden. They cause superficial gastritis, destruction of gastric parietal cells, and atrophic gastritis, resulting in reduced availability of IF for vitamin B12 transport. This causes an interference with vitamin B12 absorption, thus leading to vitamin B12 deficiency and its clinical manifestations. In genetically susceptible individuals, *H. pylori* infection triggers autoantibodies by a mechanism of molecular mimicry;[12,13] however, studies showed that only a minority of patients with PA are infected with *H. pylori*.[12,13] The effect of *H. pylori* treatment on the potential reversal of atrophic gastritis is also controversial.[14,15]

Studies to understand the genetic component of PA are long overdue and may provide important insights into its mechanism. Furthermore, rapid progress has been made in the understanding of susceptibility to a spectrum of other autoimmune diseases through genome wide association studies.

There are two key facts that confirm the existence of a genetic basis. Firsty, PA has a familial link with ≤19% of the patients having a family member with PA. On the other hand, it has been observed that the genotypes *HLA-DRB1*03* and *DRB1*04* were significantly associated with PA. These genotypes were seen in other autoimmune diseases and support the concept that autoimmunity may play a role in PA.[16-18]

Patients with PA have been shown to have two types of antibodies: one to parietal cells and the other to IF (IFA) or its binding site in the small bowel. The immune response is directed against the gastric H+/K+-ATPase, which accounts for the associated achlorhydria. This proton pump is responsible for acid secretion in the stomach and is the major protein of the secretory canaliculi of gastric parietal cells. It produces acid by secreting H+ ions in exchange with K+. The gastric H+/K+-ATPase is formed by a catalytic 100 kDa α subunit and a 60–90 kDa β subunit. The highly conserved catalytic α subunit is phosphorylated during its reaction cycles and the β subunit comprises a heavily glycosylated 35 kDa core protein. The atrophic gastritis is caused by the action of autoreactive CD4+ T cells that recognise H+/K+-ATP ase, which leads to their immune destruction.[19-21] The β subunit is considered the causal antigen and the source of the autoimmune response responsible for the damage to the gastric mucosa. Parietal cells are present at a high frequency in PA (80–90%), especially in early stages of the disease and bind to both the α and β subunits of gastric H+/K+-ATPase. In the later stages of the disease, the incidence of parietal cells decreases due to the progression of autoimmune gastritis and a loss of gastric parietal cell mass, the result of the decrease in antigenic rate.

Studies have reported IFA positivity in 40–60% of patients with PA. These antibodies lead to cobalamin malabsorption in the terminal ileum that leads to cobalamin deficient megaloblastic PA.[22,23] However, iron deficiency anaemia, a known complication of achlorhydria, occurs predominantly in women and precedes the onset of cobalamin-deficient PA by approximately 20 years.[19,24] So, the patients with unexplained iron deficiency anaemia should be checked for autoimmune gastritis and PA.

CLINICAL FEATURES

PA usually manifests itself in people >30 years old (usually adults >60 years) and affects both sexes equally. The clinical presentation proceeds gradually, and patients usually exhibit symptoms of anaemia with pallor, fatigue, lightheadedness, or tachycardia and decreased mental concentration. Involvement of small-bowel epithelium may result in malabsorption and diarrhoea, with weight loss and anorexia being additional common complaints. Glossitis is a frequent sign of megaloblastic anaemia, with the patient displaying a painful, smooth, red tongue. Other symptoms reported include dyspeptic symptoms, epigastric discomfort, postprandial bloating and fullness, and early satiety.

The elevation in bilirubin levels, caused by ineffective erythropoiesis, manifests as jaundice. Patients may develop neurological symptoms or may be associated with autoimmune diseases such as autoimmune thyroid disease, Type 1 diabetes mellitus, and vitiligo, as part of the autoimmune polyendocrine syndromes.[3,4,23,25,26]

NEUROLOGIC ABNORMALITIES

Neurologic abnormalities are seen in PA as a result of vitamin B12 deficiency. It could be isolated or be the first manifestation of vitamin deficiency and occur without any haematological or gastrointestinal context. Demyelination is the initial finding, which progresses to axonal degeneration and neuronal death if left untreated. The widely known major manifestations described include peripheral neuropathy, subacute combined degeneration of spinal cord, dementia, ataxia, optic atrophy, psychosis, and mood disturbance. Further neurological disorders described include cerebellar ataxia, abnormalities of cranial nerves, parkinsonism, and movement disorders.[26-31]

Isolated cases of nominal dysphasia or amnesic aphasia were reported.[32,33] A minority of patients exhibit mental or psychiatric disturbances (psychosis) or autonomic signs (bladder, erectile dysfunction, and orthostatic hypotension). Additionally, patients with PA must be closely observed for hypotension and it is also advisable to screen patients with chronic postural hypotension for vitamin B12 deficiency.[34] Epilepsy is rarely seen in adult cases. The appearance of motor symptoms is indicative of subacute combined degeneration involving the dorsal and lateral spinal columns. Imaging of the spinal cord in cases of severe myelopathy that are not initially recognised as the result of vitamin B12 deficiency, had characteristic hyperintensity on T2-weighted imaging, described as an inverted V-shaped pattern in the cervical and thoracic spinal cord.[28,29] It is particularly important to recognise these symptoms early because the neurological lesions may not be reversed after replacement therapy with vitamin B12.

GASTRIC CANCER AND PERNICIOUS ANAEMIA

Patients with PA may also be at a higher risk for developing gastric cancer (GC) (adenocarcinoma and gastric carcinoid Type I) as an end-stage evolution of atrophic gastritis. Hypergastrinaemia, secondary to hypochlorhydria in PA patients, is a well-known risk factor for enterochromaffin-like cell hyperplasia and gastric carcinoids. Hypochlorhydria leads to overgrowth of nitrosamine producing bacteria with potential carcinogen activity.[35] However, more extensive atrophy of the gastric mucosa (multifocal atrophic gastritis) seems to be associated with an increased risk of progression to gastric neoplastic lesions.[35]

The meta-analysis presented by Vannella et al.[36] reported that PA is associated with a nearly 7-fold relative risk of GC and the incidence-rate of GC in PA is 0.27% per person-years. Furthermore, a recent meta-analysis by Lahner et al.[37] showed an overall lower relative risk of cancers other than GC in PA patients, but an increased relative risk of biliary tract cancers and haematological malignancies was observed. The increased risk for the development of gastric neuroendocrine tumours in patients with PA represent an additional potential rationale for endoscopic surveillance in these patients.[38] The management of patients with PA remains controversial and the need for endoscopic and histological surveillance strategies to prevent GC in these patients is not universally accepted. However, in young patients, and when endoscopy detects any preneoplastic characteristic lesions, most experts agree that it is convenient to perform an endoscopic evaluation at the diagnosis of PA and then every 2–5 years. Additionally, the British Society of Gastroenterology (BSG) guidelines recommended a follow-up with endoscopic surveillance every 3 years to patients with extensive GA. PA patients should be monitored regularly using gastroscopy with antral and corporal biopsies.[39]

PERNICIOUS ANAEMIA PRESENTING WITH HYPERHOMOCYSTEINEMIA ASSOCIATED THROMBOEMBOLISM

Several case-control studies and even a meta-analysis have confirmed a link between venous thrombosis and hyperhomocysteinemia. Homocysteine is due to genetic and acquired factors (poor diet in folate and vitamin B12, older age, renal impairment, thyroid diseases, and malignancies) induced by the intake and the concentrations of vitamin B9 or B12 in the majority of cases.[40,41] However, the most common cause of vitamin B12 deficiency with hyperhomocysteinemia is PA.

It was thought that the main pathophysiological link among these vitamins and venous thrombosis is the accumulation of homocysteine as a result of decreased concentrations of these B group vitamins. However, all of these vitamins have a homocysteine-independent role related to the development of venous thrombosis. In addition, hyperhomocysteinemia inhibits the inactivation of factor Va by activated protein C and could increase the effect of factor V Leiden. Many hypotheses have been suggested to explain how hyperhomocysteinemia may lead to venous thrombosis. One hypothesis is that homocysteinemia has a toxic effect on the vascular endothelium and on the dotting cascade.[40] Addittionally, homocysteine has several procoagulant properties including the decrease of antithrombin III binding to endothelial heparan sulfate, increase of affinity between lipoprotein(a) and fibrin, induction of tissue factor activity in endothelial cells, and inhibition of inactivation of factor V by activated protein C.[42,43] Understanding the molecular pathogenesis of the development of thrombosis in patients with hyperhomocysteinemia related to Biermer's disease would help us identify patients at risk and treat them accordingly. Thus, these conditions should remain in the clinician's mind, especially when thrombosis occurs along with biological abnormalities such as anaemia, megaloblastosis, or haemolysis.[44]

DIAGNOSIS OF PERNICIOUS ANAEMIA

The diagnosis of PA relies on the presentation of megaloblastic anaemia, low serum vitamin B12 levels, gastric atrophy, and the presence of antibodies to gastric parietal cell or IF (Figure 1).

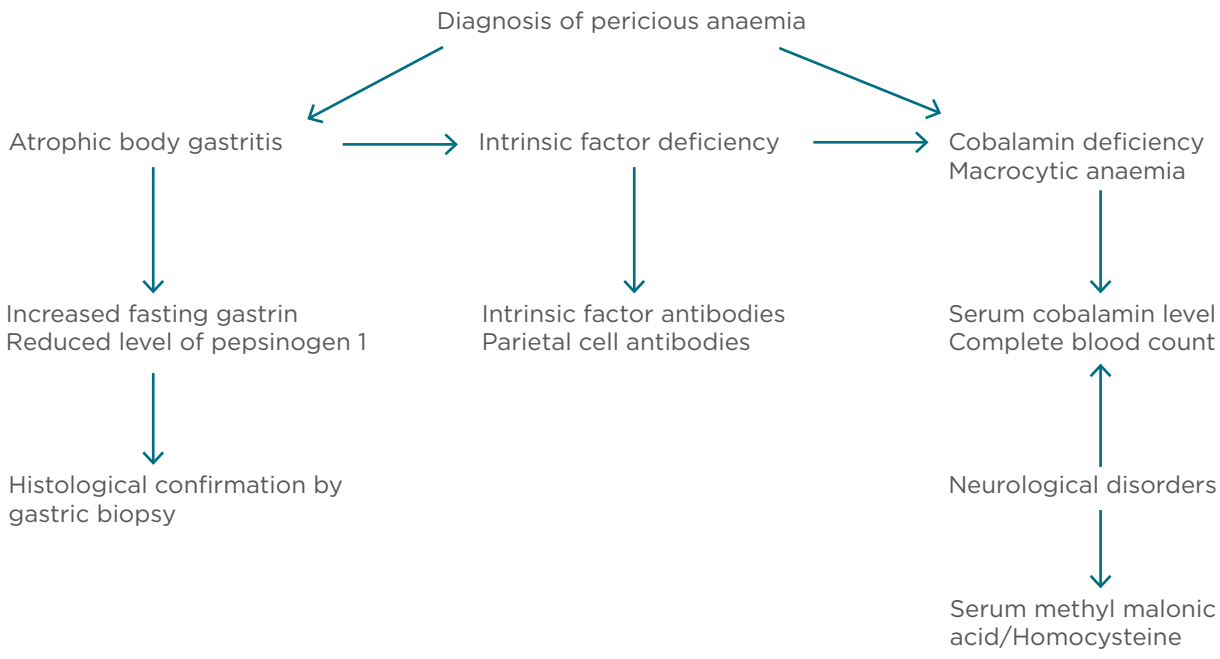

Figure 1: Diagnostic algorithm for pernicious anaemia.[8,50]

The anaemia is macrocytic and normochromic with a reduction in the absolute number of reticulocytes. The patient's red blood cells exhibit marked anisopoikilocytosis and numerous oval macrocytes. Although the hypersegmented neutrophils support the diagnosis of megaloblastic anaemia, they are not specific. The variation in the size and shape of red blood cells could lead to a misdiagnosis of microangiopathic haemolytic anaemia instead of megaloblastic anaemia. Some patients may present with nonanaemic macrocytosis for several months before the diagnosis of PA is made. Additionally, macrocytosis is absent in 30% of PA patients if iron deficiency is associated. Moreover, there may be masking of macrocytosis by α-thalassemia in PA patients with African origins who might present with microcytosis. Pancytopenia is often present with rates ranging from 5 to 37%.[45]

Schistocytes may be seen in megaloblastic anaemias as a reult of erythroblast cytoskeletal fragility, reflecting the severity of dyserythropoiesis. Several cases of PA presenting with pseudothrombotic microangiopathy are reported in the literature and treated with plasma transfusions and exchange. These cases are characterised by haemolysis, thrombocytopenia, and schistocytosis with higher mean LDH levels.[46] Very high LDH levels, mild-moderate thrombocytopenia, and a low reticulocyte count are strongly suggestive of pseudothrombotic microangioapthy and should prompt the physician to screen for cobalamin deficiency.[47]

Bone marrow biopsy and aspiration are not necessary for the diagnosis of megaloblastic anaemia (Figure 2) and may be misleading in cases of severe pancytopenia with hypercellularity, increased erythroblasts, and even cytogenetic abnormalities, confusing the diagnosis with acute leukaemia. It shows a hypercellular bone marrow with a shift toward immaturity and abnormal maturation of erythroid and myeloid cell lines. The immature neutrophil series exhibits nuclear-cytoplasmic asynchrony with numerous giant metamyelocytes. The ineffective erythropoiesis and myelopoiesis are responsible for the pancytopenia in megaloblastic anaemia, despite marrow hypercellularity.[3,4]

Serum vitamin B12 and serum folate levels should be determined concurrently to correctly identify patients deficient in either or both; however, there exists limited sensitivity and specificity.

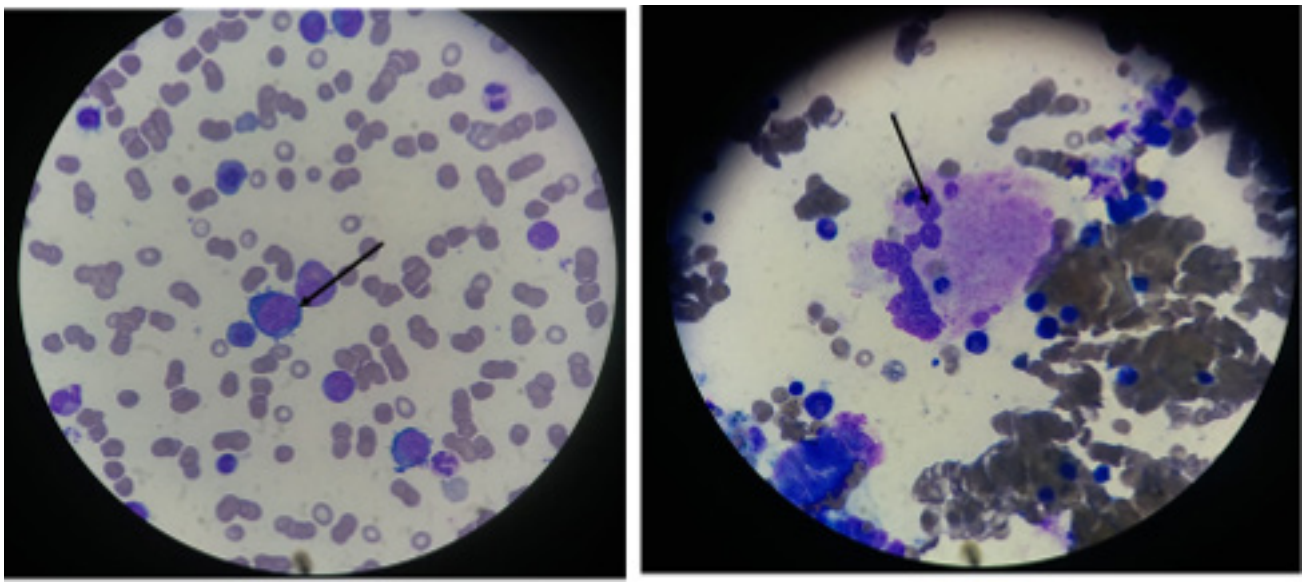

Figure 2: Bone marrow biopsy displaying megablastosis.

A bone marrow aspirate shows megaloblastic features. Large erythroblasts and other red-cell precursors are characterised by an open, immature nuclear chromatin pattern. There is dyssynchrony between the maturation of the cytoplasm and that of the nuclei in later red-cell and granulocyte precursors.

Photographs courtesy of Dr Benkirane.

Differentiating between vitamin B12 deficiency and folate deficiency is essential to patient management because treatment of vitamin B12 deficient patients with folate alone may reverse the megaloblastic blood picture, although the associated neurologic damage may worsen. Cobalamin level is measured by an automated competitive-binding immunoenzymatic luminescence method, the results of which may not always accurately reflect actual vitamin B12 stores. Low levels (<100 pg/mL) are usually associated with clinical deficiency, however both false-negative and false-positive values are common (occurring in up to 50% of the tests), attributable to the fact that only 20% of the total measured vitamin B12 is on the cellular delivery protein TC; the remainder is bound to haptocorrin, a protein of unknown function. IF antibodies may bind the test IF reagent and if there is a failure in the denaturation step intended to denature IF-blocking antibodies, spuriously normal or increased vitamin B12 levels can be measured.[48,49] In this situation, measurement of serum methylmalonic acid and total homocysteine is useful in making the diagnosis of vitamin B12 (markedly elevated levels) deficiency in patients who have not received treatment, including those who have only neurologic manifestations of deficiency.

ELEVATED LEVELS OF TOTAL HOMOCYSTEINE AND METHYLMALONIC ACID

Methylmalonic acids have been proven as markers for insufficient intracellular vitamin B12 because the levels can be remeasured to document adequate vitamin B12 replacement. An elevated level of methylmalonic acid is reasonably specific for vitamin B12 deficiency, and the level always decreases with vitamin B12 therapy. The level of serum total homocysteine is less specific because it is also elevated in folate deficiency, classic homocystinuria, and renal failure.

A deficit of IF may be demonstrated using the Schilling test, a dynamic multistep investigatory test which involves the ingestion of isotope-labelled vitamin B12, followed by an injection of unlabelled vitamin B12. Given its complexity and problems related to the use of radioactive agents, the Schiling test is now being replaced by other diagnostic strategies such as the detection of IFA.

A positive test for anti-IFA or anti-parietal-cell antibodies (by immunoblotting, ELISA, and chemiluminescent immunoassay methods) identifies an autoimmune basis for the gastric atrophy in PA. Anti-parietal-cell antibodies are found in 90% of patients with PA, but have low specificity and are seen in atrophic gastritis without megaloblastic anaemia as well as in various autoimmune disorders. IFA are less sensitive as a result of only being found in 60% of patients with PA, but are considered highly specific for PA. However, a positive correlation between the increasing histological score of body mucosa atrophy and the titer of both antibodies can be observed.[18,39] Surveillance for autoimmune thyroid disease is reasonable in patients with positive antibody tests. A diagnostic workup of megaloblastic anaemia should also include evaluation of iron because the bone marrow is overloaded with iron that cannot be utilised during the megaloblastic state. Therefore, iron supplementation may be warranted even though the patient has an initial normal serum iron value.

Chronic atrophic gastritis can be diagnosed on the basis of an elevated fasting serum gastrin level and a low level of serum pepsinogen I. Some experts recommend endoscopy to confirm gastritis and rule out gastric carcinoid and other GC because patients with pernicious anaemia are at increased risk for such cancers. In PA patients, the mucosa of the cardia and fundus is thinned and atrophied, with shrunken glands and containing few principal and parietal cells, while usually the mucosa of the antrum is spared. However, a concomitant antral atrophic gastritis may be observed in 25% of PA patients.[50] These data strongly suggest that an extension of gastritis to the gastric antrum does not necessarily exclude the diagnosis of PA and the presence of gastric autoimmunity.

Differential Diagnosis

Accurate differential diagnosis of other causes of macrocytic anaemia and cobalamin deficiency is mandatory (Table 1).

Table 1: Other causes of macrocytic anaemia and cobalamin deficiency.[3,4]

Causes of macrocytic anaemia	Causes of cobalamin deficiency
Hypoplastic anaemia, myelodysplastic syndrome	Total or partial gastrectomy Gastric bypass or other bariatric surgery Ileal resection or organ reconstructive surgery
Folate deficiency	Corpus-predominant *Helicobacter pylori* gastritis
Liver disease (alcoholic, advance cirrhosis, poor dietary intake)	Inflammatory bowel disease, tropical sprue
Haemolytic anaemia, response to haemorrhage	Imerslund–Gräsbeck and other syndromes
Drugs (e.g., methotrexate, azathioprine, 6-mercaptopurine, acyclovir, 5-florouracil, phenobarbital)	Protein-bound vitamin B12 malabsorption
Chronic obstructive pulmonary disease	Mild atrophic gastritis
	Use of metformin or drugs that block stomach acid
	Vegan of vegetarian diet, or diet low in meat and dairy products

TREATMENT

The clinical management of patients with PA has two different aspects: the treatment of cobalamin deficiency and the monitoring of iron deficiency onset. PA is caused by inadequate secretion of gastric IF, which is necessary for vitamin B12 absorption and thus cannot be treated with oral vitamin B12 supplements.

The therapeutic recommendations for PA, with regard to dosage and administration of vitamin B12 substitution treatment, are divergent. Vitamin B12 must be administered parenterally and patients generally receive an intramuscular injection of 1,000 µg B12 every day or every other day during the first week of treatment. The next month, they receive injections every week, subsequently followed by monthly injections.

The alternative to intramuscular B12 injection is high-dose oral B12, to which a 1,000–2,000 µg/day dose has been demonstrated to be effective.[51] However, despite many studies suggesting oral administration of vitamin B12 to be easy, effective, and less costly than intramuscular administration, debate surrounds the effectiveness of the oral route. Patients should be offered this alternative after an informed discussion on the advantages and disadvantages of both treatment options. The effect of oral cobalamin treatment in patients presenting with severe neurological manifestations has not yet been adequately documented. Although, recommendations are to always use the parenteral route in severe neurological manifestations. Approved sublingual and intranasal formulations of B12 are also available.[52,53]

PA requires lifelong treatment and the percentage of vitamin B12 absorption improves with supplementation, but symptoms of vitamin B12 deficiency may be improved after just a few days of medical treatment. Gastric atrophy, however, is not cured by cobalamin.

MONITORING

The earliest sign of treatment response is an increase in reticulocyte count, usually within 3 days of treatment. Following changes in the decrease of biochemical markers such as methylmalonic acid and plasma homocysteine levels have been observed in the first 5 days of treatment.[4,8] Sustained normalisation of serum cobalamin usually occurs following 2 weeks of therapy.

The macrocytosis correction takes place during the first month of treatment. The surveillance of these patients is mandatory to detect early long-term consequences of PA, such as GC and carcinoids.[50] A clinical interview should be considered every year to attest the

commencement of new symptoms. These may include epigastric pain, dysphagia, iron deficiency, and/or others that require gastroscopic investigation. The key management principle is the importance of routine follow-up.

CONCLUSION

PA is an underdiagnosed autoimmune disease. It is a complex disorder consisting of haematological, gastric, and immunological alterations. Macrocytic anaemia is the result of vitamin B12 deficiency, which, in turn, is the result of deficiency of IF corpus atrophy. The diagnosis of PA remains challenging in many circumstances for many clinicians because of its diverse clinical manifestations and the limitations of currently available diagnostic tools. Early detection and treatment have led to a lower percentage of vitamin B12 deficiency patients with PA.

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‘Girdle Syndrome’ Progressing to Ischaemic Colitis and Acute Intrahepatic Cholestasis in a Patient with Sickle Cell Disease: A Case Report

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Abstract

In this paper, the case of a 34-year-old male with sickle cell disease, recurrent episodes of ‘girdle syndrome’, and development of chronic ischaemic colitis is reported. At his last admission to the hospital, he presented with ileus attributed to severe intestinal ischaemia. During his hospitalisation, despite optimal supportive treatment, he developed acute liver failure, possibly as a result of acute intrahepatic cholestasis, a rare but fatal complication of sickle cell disease, and died from sepsis and multiorgan failure.

INTRODUCTION

Acute vaso-occlusive crisis and haemolysis characterise sickle cell disease. Rarely observed, ‘girdle syndrome’ is a situation in which vaso-occlusion occurs in the lungs, liver, and mesentery, and the pain has a characteristic girdle-like distribution. The clinical appearance is nonspecific, with a wide range of manifestations ranging from abdominal pain without significant findings upon physical examination, to ischaemic colitis with a clinical picture of acute abdominal pain and symptoms of ileus and/or colonic perforation. Girdle syndrome is more common in children and, although rare, is more likely to develop during the course of an acute, painful sickle crisis. The authors describe the case of a 34-year-old male who was admitted with girdle syndrome and developed chronic colitis and intrahepatic cholestasis. Acute abdominal pain crises often present with a complicated clinical picture and diagnostic dilemma because it can be difficult to distinguish typical vaso-occlusive pain episodes from their complications and other causes of abdominal pain.

CASE REPORT

A 34-year-old male patient with sickle cell anaemia was admitted to the emergency ward of the author’s institution because of right upper quadrant abdominal pain which started a

few weeks before; moreover, the patient noted diarrhoea, worsening of appetite, and fatigue. Physical examination demonstrated jaundice, right upper quadrant tenderness, an enlarged liver, as well as a distended abdomen. He had a history of recurrent pain crises and was submitted to cholecystectomy a few years before because of the presence of gallstones. Laboratory evaluation revealed a white cell count of 14.32x10^9/L, haemoglobin of 7.60 g/dL, and a platelet count of 515.00x10^9/L. Serum chemistry showed potassium of 3.8 mmol/L, blood urea nitrogen of 17.0 mg/dL, and creatinine of 0.7 mg/dL. There was a slight elevation of aspartate aminotransferase (49.0 U/L) and lactate dehydrogenase (313.0 U/L), and a marked elevation of total bilirubin (3.6 mg/dL), alkaline phosphatase (410.0 U/L), and γ-glutamyltransferase (563.0 U/L) with normal amylase levels (37.0 U/L). International normalised ratio (INR) was 1.3, prothrombin time was 15 seconds, and activated partial thromboplastin time was 31 seconds. C-reactive protein concentration was 40.30 mg/L. He was found to be a carrier of hepatitis B virus, with undetectable serum hepatitis B virus-DNA levels. He was negative for hepatitis C antigen and positive for IgG antihepatitis A antibodies, and serology tests for cytomegalovirus and herpes simplex virus-1 and 2 were negative. The abdominal ultrasonography showed hepatomegaly with diffuse increases in liver echogenicity without ascites or features of cirrhosis. No gallstones were seen, and the spleen was atrophic, indicating autosplenectomy. A barium enema examination showed thumbprinting in the caecum and ascending colon. A rectal swab culture was obtained and was negative for enteric pathogens. The patient was hospitalised and treated with red blood cell exchange transfusions, analgesics, and intravenous metronidazole and ciprofloxacin. The patient showed improvement and left the hospital after 6 days. Considering the risk of the patient already having a hypercoagulable disorder, they were tested for all five inherited thrombophilias (mutations in the genes that encode protein S, protein C, antithrombin, factor V Leiden, and prothrombin), and for antiphospholipid syndrome, for which no pathological findings were revealed.

In the following months, the patient had repeated hospital admissions for pain crises, abdominal pain, diarrhoea, and weight loss. For every admission he was treated with red blood cell exchange transfusions, intravenous hydration, antibiotics, and analgesics; haemaglobin S levels were maintained at <28%. A lactose-free diet was recommended, but the patient continued to have six to eight loose stools per day. A flexible rectosigmoidoscopy revealed areas of exudate and necrosis in the rectosigmoid colon with multiple blood clots, and biopsies obtained did not show any significant findings. Stool cultures and stool parasitology tests were negative. Abdominal T2-weighted MRI to assess iron overload showed no signs of secondary liver haemochromatosis, with liver iron being 1.1 mg/g (dry liver measurement), but significant liver hypertrophy and upper abdominal varices were suggestive of portal hypertension. CT evaluation of the abdomen revealed bowel distention, thickening of the intestinal wall, mesenteric lymph node enlargement, signs of liver cirrhosis, and presence of ascites. Liver elastography showed an elevated liver stiffness of 13.2 kPa, indicative of liver cirrhosis.

On the patient's last admission, he had developed serious diarrhoea, of which manifested as >20 loose stools per day, and his abdomen became distended with generalised tenderness and hypoactive bowel sounds. An abdominal radiogram showed elevation of the right hemidiaphragm as a result of the enlarged liver, dilated loops of small bowel but no air-fluid levels (Figure 1). The serum amylase, lipase, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase were markedly elevated. A CT scan revealed bowel distention, thickening of the intestinal wall, thumbprinting, and a moderate volume of ascitic fluid (Figure 2). Multiple liver infarcts appeared as circumscribed, peripheral, wedge-shaped areas of decreased attenuation. A peritoneal tap yielded a cloudy fluid which was positive for *Escherichia coli*. After 2 weeks of being admitted to hospital, the patient began to develop an abrupt onset of jaundice elevation and liver dysfunction with subsequent development of encephalopathy and coagulopathy. Laboratory evaluation revealed a white cell count of 6.34x10^9/L, haemoglobin of 7.40 g/dL, and a platelet count of 158.00x10^9/L. Serum chemistry showed potassium of 5.2 mmol/L, blood urea nitrogen of 169.0 mg/dL, and creatinine of 1.2 mg/dL. There was a slight elevation of aspartate aminotransferase (56.0

U/L), alanine transaminase (52.0 U/L), and lactate dehydrogenase (326.0 U/L), as well as a marked elevation of total bilirubin (15.1 mg/dL), alkaline phosphatase (329.0 U/L), and γ-glutamyltransferase (495.0 U/L). C-reactive protein concentration was 29.4 mg/L and INR was 2.4. The patient also developed haematochezia, a tense and tender abdomen, and the absence of bowel sounds. Oral feeding was discontinued, and the patient was placed on parenteral nutrition. The patient required multiple transfusions of packed red blood cells and fresh frozen plasma because of severe reductions in procoagulant and anticoagulant factors, and a dopamine drip for hypotension. Stool examination was negative for *Clostridium difficile* toxin and blood cultures were negative for cytomegalovirus, HIV, and *Yersinia*. A blood culture was positive for *Klebsiella pneumoniae* and therefore he was treated with colistin. Despite prudent clinical care and all applicable therapeutic interventions, the condition of the patient was irreversible, and the patient died as a result of multiorgan failure.

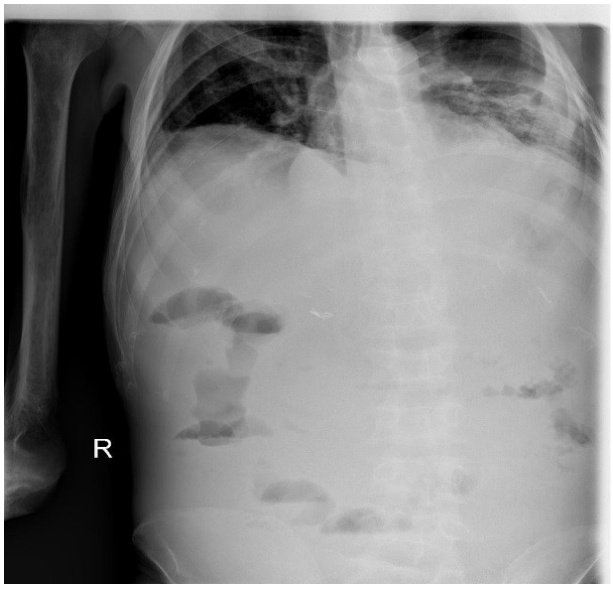

Figure 1: Abdominal X-ray showing elevation of the right haemidiaphragm as a result of the enlarged liver.

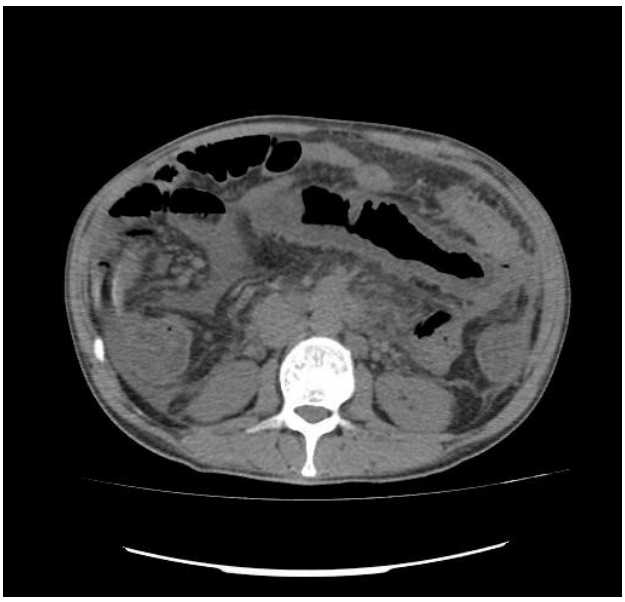

Figure 2: CT evaluation of the abdomen revealed bowel distention, thickening of the intestinal wall, and thumbprinting, indicative of ischaemic bowel disease.

DISCUSSION

Acute abdominal pain is a common symptom in patients with sickle cell disease. It is often difficult for a clinician to deduce whether it is a sickle cell crisis or another intra-abdominal pathology requiring surgical treatment; however, proper diagnosis is mandatory to not only avoid unnecessary laparotomies that can aggravate a sickle cell crisis, but to also provide the appropriate analgesic and supportive treatment.

Acute abdominal pain as a result of a sickle cell crisis is thought to be caused by vaso-occlusions in the microcirculation of the mesentery and the viscera that irritate the peritoneum. In most cases the pain is self-limiting and does not lead to ischaemic colitis.[1] A particular clinical entity with rare occurrence is mesenteric syndrome, otherwise known as girdle syndrome, presenting with vaso-occlusions in mesenteric vessels that is often associated with acute thoracic syndrome. The clinical appearance of girdle syndrome is nonspecific, with a wide range of manifestations ranging from abdominal pain without significant findings upon physical examination, to ischaemic colitis with a clinical picture of acute abdominal pain and symptoms of ileus and/or colonic perforation.[2] In most cases, the pain is accompanied by haematochezia, but without abdominal tenderness, rebound tenderness, or reductions in intestinal sounds.[3] Differential diagnosis of abdominal pain in sickle cell disease includes hepatic or splenic sequestration crisis, hepatic infarctions and acute hepatic crisis, pancreatitis, choledocholithiasis, cholecystitis, appendicitis, and stomach ulcers, occurring at an increased incidence in this population.[4-6] A complete blood count is necessary, in conjunction with biochemical control of renal and hepatic function and amylase levels, for the exclusion of these other conditions, as well as blood cross-matching for the onset of transfusion therapy. Artery blood gases and chest X-rays are useful tests to investigate the involvement of the respiratory tract in the crisis. Bowel dilatation and the thumbprint sign are features of ischaemic colitis in the abdominal radiograph and are most visible in the first 24–72 hours.[7] Significant for diagnosis is the role of CT imaging, which demonstrates thickening of the intestinal wall with narrowing of the lumen as a result of mucosal oedema. Free air may also be observed in the peritoneum in the event of perforation. Colonoscopy provides the highest diagnostic accuracy, because observation of the haemorrhagic oedematous mucosa and ulcerations characterise ischaemic colitis,[8] while microscopic vessel obstructions in the presence of sickle cells are observed in the microscope. However, colonoscopies should be performed with caution because the bowel dilatation reduces blood supply to the intestinal mucosa and may exacerbate ischaemia.[9] Conservative treatment with feeding discontinuation, analgesia, transfusion or exchange transfusion therapy, hydration, and potentially antibiotic coverage is typically sufficient, while a laparotomy is required in cases of doubt regarding diagnosis but also for the treatment of complications in persistent vascular obstruction with significant irreversible ischaemia or perforation of the intestine. During a sickle cell crisis, a laparotomy should be avoided because it leads to hypoxia which strengthens the sickling process and halts remission of the symptoms; if necessary, exchange transfusions should be preceded.

Acute intrahepatic cholestasis is the most severe acute clinical manifestation of sickle cell disease concerning the liver and, although rarely, can be fatal. It initially presents as an acute liver crisis with fever and right upper quadrant abdominal pain, and rapidly evolves into acute hepatic failure with coagulation disorders and hepatic encephalopathy. Laboratory findings are characterised by a large increase in bilirubin levels, mainly of its conjugated fraction, due to haemolysis, cholestasis, and renal failure.[10] Transaminases also markedly increase and prothrombin time, activated partial thromboplastin time, and INR are prolonged. Treatment is supportive with immediate onset of transfusions or exchange transfusions and administration of fresh frozen plasma for the treatment of coagulation disorders.[9] Renal function generally reverts after the treatment of liver insufficiency and chronic dialysis is not required: only temporary dialysis in the acute phase is required. The prognosis is often poor.[10,11]

This patient had developed chronic ischaemic colitis as a result of repeated episodes of vaso-occlusions concerning the mesenteric vessels. Despite maintaining haemoglobin S

levels <28% with exchange transfusion therapy, on his last admission he developed severe colonic ischaemia with the clinical presentation of ileus and multiple liver infarcts leading to acute liver failure, encephalopathy, and coagulopathy (i.e., severe girdle syndrome). The clinical presentation and the laboratory findings were indicative of acute intrahepatic cholestasis. Despite optimal treatment, he died from sepsis and multiorgan failure.

CONCLUSION

This is a teaching case of a patient having received all accessible treatment, but the course of the illness was catastrophic. Sickle cell disease is a benign multisystem disease mandating multidisciplinary management from an experienced medical team. The authors support the introduction of new targeted therapies to significantly reduce these complications from a benign disorder.

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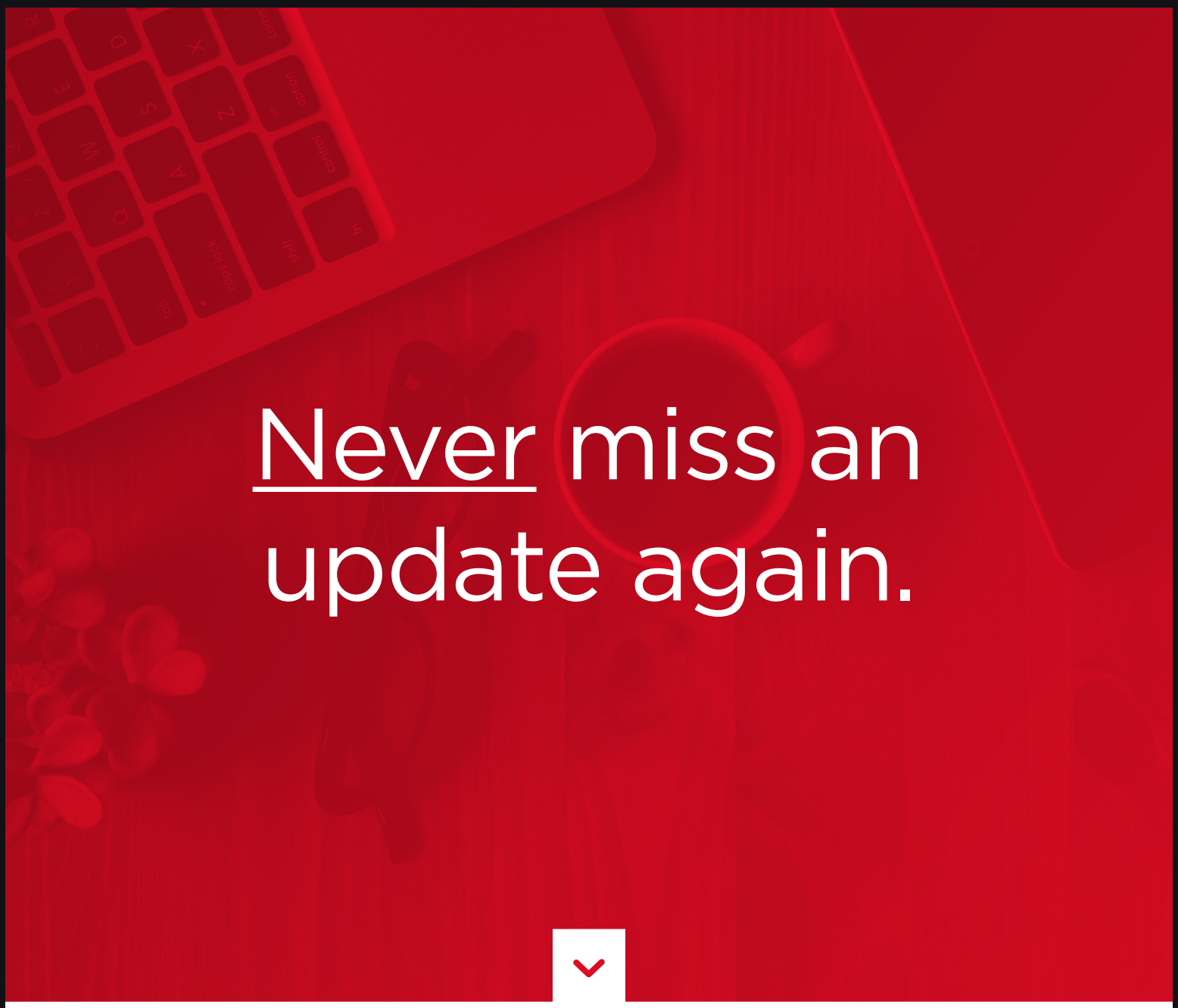

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