The Bella Tripp Foundation

Better Lungs and Life after Transplant

Progress Report 1

The Bella Tripp Foundation
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Progress Report – Better Lungs and Life after Transplant

On behalf of The Bella Tripp Foundation, I am pleased to share with you our first Progress Report: Better Lungs and Life after Transplant. This report provides a comprehensive overview of our impact and activities since the Foundation's establishment in August 2023.

We are profoundly grateful for your contribution to this progress. Your support—whether through time, funding, collaboration or awareness—has been instrumental in helping us work toward a future where no child is lost to undiagnosed post-transplant respiratory complications. Please find the report enclosed for your review. We hope it offers insight into the difference your involvement is making. If you have any questions or would like to discuss how we can continue working together, I warmly welcome the opportunity.

Thank you for standing with us as we continue this journey. We look forward to building on this momentum in the years ahead.

Kind Regards,

Yasmina Tripp - Chair

Tina Tripp - Director

Emma Auld - Director

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Thank You From Our Founder

As I reflect on the journey of The Bella Tripp Foundation to date, I am deeply moved by the generosity, dedication, and compassion of those who have stood with us. This Foundation was born out of love—a love for a remarkable young girl whose kindness knew no bounds. Established in August 2023 in memory of my daughter, Isabella Claire Tripp—Bella—our mission is to honour her legacy by advancing research and awareness to improve outcomes for children facing similar medical challenges.

Bella's passing in April 2022, at just 14 years old, was a profound loss. She faced leukaemia with extraordinary courage, but complications from a bone marrow transplant ultimately took her life. Among the greatest risks for children undergoing these transplants are respiratory complications, which often go undetected until it is too late. Our Foundation is dedicated to changing that.

> "We have worked tirelessly to raise awareness and secure funding for critical research into early detection and treatment of post-transplant lung diseases."

Since our inception, we have worked tirelessly to raise awareness and secure funding for groundbreaking research into the early detection and treatment of post-transplant lung diseases. Over the past year, we have expanded our efforts, deepening our commitment to funding critical research, engaging with the community to drive awareness, and strengthening our advocacy through meaningful partnerships. By funding research focused on early detection of potential complications, we are working towards improved treatment options that could save lives. Through community engagement, we are ensuring that families, healthcare professionals, and policymakers recognise the urgency of this issue.

And by collaborating with leading researchers and institutions, we are helping to accelerate medical advancements in this field.

> "Every contribution, every event, and every partnership has helped us move closer to our vision: a future where children who undergo bone marrow transplants have a real chance at a healthier life."

I am profoundly grateful for the unwavering support from our community—our donors, volunteers, medical professionals, and advocates. Every contribution, every event, and every partnership has helped us move closer to our vision: a future where children who undergo bone marrow transplants have a real chance at a healthier life.

As we look ahead, we remain steadfast in our mission. There is still much to do, but together, we can continue to create lasting change. Thank you for being part of this journey of love, resilience, and hope.

Yasmina Tripp
Founder and Chair,
The Bella Tripp Foundation

Our Vision and Mission

At The Bella Tripp Foundation, our mission is to drive research that leads to the early diagnosis and treatment of lung diseases in children who have undergone bone marrow transplants. By securing funding and fostering partnerships with leading medical institutions, we are working towards a future where no child's life is lost due to undetected post-transplant complications. Alongside research, we are dedicated to raising awareness and advocating for systemic improvements in paediatric bone marrow transplant care, ensuring that families and healthcare providers have access to the knowledge and resources needed to improve outcomes.

Our vision is a world where every child who undergoes a bone marrow transplant has access to early detection and effective treatment for respiratory complications. Through research, education, and advocacy, our vision will be achieved when the risk of fatalities or long-term negative health outcomes arising from bone marrow transplant associated respiratory complications and disease has been completely removed.

Did you know?

Bone marrow diseases can be caused by genetic factors, environmental exposures, or other underlying health conditions.

Treatments may include medications, blood transfusions, or bone marrow transplants. [1]

Younger donors (18-30 years old) are most needed for bone marrow donations.

This is because their blood stem cells result in the best outcomes for transplant patients. [2]

A staggering 52% of children who have a bone marrow transplant (BMT) experience respiratory complications

Children who develop such complications have nearly a fivefold increased risk of mortality within five years post-transplant compared to those without complications. [3]

Sources:
[1] MedlinePlus. Bone Marrow Diseases. U.S. National Library of Medicine.
[2] Leukaemia Foundation. Blood Stem Cell (Bone Marrow) Donation.
[3] Weiner, D. J., et al. "Lung Function, Pulmonary Complications, and Mortality after Allogeneic Blood and Marrow Transplantation in Children." Biology of Blood and Marrow Transplantation, vol. 15, no. 7, 2009, pp. 817–826.

More About Us

WHO WE ARE

The Bella Tripp Foundation is an Australian registered not-for-profit organisation dedicated to improving survival outcomes for children undergoing bone marrow transplants. Established in August 2023 in loving memory of Isabella Claire Tripp, Bella for short, the Foundation is committed to funding vital research, raising awareness, and advocating for early detection and treatment of post-transplant lung diseases. By working closely with medical institutions, researchers, and healthcare professionals, we aim to drive advancements that will make a meaningful difference in the lives of children facing similar medical challenges.

OUR STORY

Bella was a bright, kind-hearted, and courageous young girl who faced leukaemia with unwavering strength. In April 2022, at just 14 years old, she tragically passed away due to respiratory complications following a bone marrow transplant. These complications remain a leading cause of post-transplant mortality, often undetected until it is too late.

Her legacy lives on through the Foundation, which strives to ensure that children undergoing similar medical journeys receive the best possible care and support. Through research, advocacy, and awareness, we are determined to create a future where early detection and intervention can prevent such tragedies.

> “Through research, advocacy, and awareness, we are determined to create a future where early detection and intervention can prevent such tragedies.”

WHAT WE DO

The Foundation actively funds medical research focused on early detection and treatment of post-transplant lung disease. By working with researchers and clinicians, we help drive progress in developing diagnostic tools and treatment options that can save lives.

Raising awareness is another key aspect of our work, ensuring that families, medical professionals, and policymakers recognise the urgency of this issue. Advocacy remains at the heart of our efforts as we continue to push for medical advancements and policy changes that improve the standard of care for paediatric transplant patients.

OUR IMPACT SO FAR

To date, we have:

- funded initial stages of critical medical research;
  - held numerous fundraising and awareness raising activities, reaching an Australia wide audience;
  - registered the Foundation with the Australian Charities and Not for Profits Commission, obtained deductible gift recipient status, and established the Foundation's board and constituent documents;
- joined ambassadors and sponsors to our cause and developed our branding and media presence;

Every milestone achieved is a step towards our goal of preventing tragedies like Bella’s from happening to other families.

LOOKING FORWARD

As we move forward, our focus remains on strengthening our impact through research, collaboration, and awareness. We will continue working closely with the medical community to fund innovative research that can change lives. Advocacy efforts will see the Foundation continue to engage with medical researchers to push for improved access to early diagnosis and treatment options.

Most importantly, we remain committed to working on solutions to reduce lung related risks for families navigating the difficult bone marrow transplant journey, offering them hope and a vision for a healthier future. With your continued support, we will keep pushing towards a world where no child’s life is lost due to undiagnosed post-transplant complications.

> “Thank you for being part of this journey of love, resilience, and hope.”

ADVOCACY AND AWARENESS

At the heart of The Bella Tripp Foundation is a simple yet powerful belief: awareness drives change. Inspired by Bella's journey, we are committed to shining a light on the respiratory complications that can arise following paediatric bone marrow transplants. The more awareness we raise, the greater our prospects are of generating further funding to continue meaningful research.

> At the heart of The Bella Tripp Foundation is a simple yet powerful belief: awareness drives change.

We invite you to be part of this change. By increasing awareness, we can help ensure that the need to find improved diagnostic tests and treatment options for respiratory complications in paediatric bone marrow transplant recipients receives greater attention.

Together, we can make a lasting impact. If you would like to support our work as an ambassador or sponsor, please contact us at info@thebellatrippfoundation.org.au.

VOLUNTEERING AND FUNDRAISING

Community involvement is at the heart of our fundraising efforts. Whether through volunteering at our events or organising your own, every contribution helps raise awareness and fund vital research into respiratory complications following paediatric bone marrow transplants.

From hosting a movie or karaoke night to holding a bake sale, sausage sizzle, fun-run, or community event, there are many ways to get involved and make a meaningful impact.
We are always grateful for those who choose to support us through fundraising. To help make your event a success, we can provide promotional materials, logos, and event publicity, and where possible, attend in person to share more about the Foundation's mission. Every effort, no matter how big or small, plays a vital role in furthering Bella's legacy and advancing life-changing research.

OUR SPONSORS

The Bella Tripp Foundation is deeply grateful for the generous support of our sponsors—Melbourne Storm, Lotus Living, Craig Whyte and Argyll Partners Chartered Accountants, White Top Events, and Made Agency. Through their ongoing support, these organisations play a crucial role in advancing medical understanding and improving outcomes for future transplant recipients. Their partnership reflects a shared dedication to honouring Bella's legacy and working towards a future where no child faces these challenges alone.

We extend our heartfelt appreciation to our sponsors for standing with us in our mission to fund life-changing research.

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Cameron Munster

Ryan Papenhuyzen

Nelson Asofa-Solomona

Glen Boss

Angus Sampson

OUR AMBASSADORS

The Bella Tripp Foundation is privileged to have the support of an inspiring group of ambassadors who are personally committed to our cause. Each ambassador has a personal connection to Bella and has seen and felt the impact that these complications can have on the life of a child as well as their family.

Our ambassadors — elite athletes Cameron Munster, Ryan Papenhuyzen, Nelson Asofa-Solomona, Glen Boss, and renowned actor Angus Sampson — use their platforms to amplify our message, advocate for better support systems, and engage in fundraising efforts. Their dedication helps shine a light on the challenges faced by transplant recipients and their families, ensuring that no child faces this journey alone.

Through their influence, generosity, and unwavering commitment, our ambassadors play a vital role in driving change and keeping Bella's legacy alive. We are deeply grateful for their support in helping us create a future where more children can thrive after life-saving transplants.

> "Their dedication helps shine a light on the challenges faced by transplant recipients and their families, ensuring that no child faces this journey alone."

Our Impact and Research Initiatives

The Bella Tripp Foundation has played a material part in advancing research, raising awareness, and fostering collaboration to improve outcomes for children undergoing bone marrow transplants. As reported in our 2023-2024 Annual Information Statement lodged with the Australian Charities and Not for Profits Commission, as at the end of 30 June 2024, we have raised $431,774.00 in funding. At the time of this report, our accounts for the 2024-2025 financial year were not finalised, however we anticipate we will maintain a modest net surplus that will be applied in subsequent reporting periods to research and awareness efforts. More detail in relation to our 2023-2024 Annual Information Statement and the Australian Charities and Not for Profits Commission regulatory regime is available at https://www.acnc.gov.au/".

Through this funding, have been fortunate to be able to make a financial contribution to 2 research papers aimed at developing improved detection methods and treatment options for post-transplant respiratory complications.

A copy of two of these papers is set out in the Appendix to this report, and a summary of one report is set out on page 16.

Also, through public campaigns and fundraising events, we have increased awareness of this critical issue, ensuring that more families, medical professionals, and policymakers recognise the urgency of early intervention.

Our five community engagement events have strengthened partnerships between researchers, healthcare professionals, and donors, creating new opportunities for collaboration and support.

See page 48 for more details about the events. These efforts are not just about funding research; they are about bringing hope.

By driving progress in medical science and advocacy, we are working towards better treatment protocols, improved survival rates, and a future where no child's life is cut short due to undiagnosed post-transplant complications.

Understanding Respiratory Complications of Bone Marrow Transplants

RESPIRATORY BURDEN OF PAEDIATRIC BMT

Up to 74% of children receiving BMT—around 5,000 children worldwide each year—experience respiratory complications. These complications often lead to serious outcomes, including reduced exercise capacity, prolonged hospital stays, oxygen dependency, and a significantly increased mortality risk within 10 years post-transplant.

While respiratory complications following BMT are common, they remain poorly understood. Early diagnosis is difficult due to limited diagnostic tools, and current treatments are often ineffective or carry significant side effects.

> “While respiratory complications following BMT are common, they remain poorly understood.”

Researchers at the Murdoch Children’s Research Institute (MCRI) and clinicians at the Royal Children’s Hospital (RCH) are committed to addressing this critical gap. Their work focuses on advancing understanding, improving early diagnosis, and developing new, effective treatments.

LEVERAGING SINGLE-CELL TECHNOLOGY

Single-cell technology allows us to study cell behaviour at an unprecedented level, revealing how specific cells contribute to disease. The teams at MCRI and RCH have launched a pilot study using this technology to analyse blood and lung samples from children who have undergone a BMT, deepening our understanding of respiratory complications at a cellular level.

The study includes a retrospective analysis of 117 pre-transplant samples and a prospective study using new samples collected during routine care, minimising discomfort.

The researchers are examining cytokines—proteins released by cells that can cause severe, irreversible lung damage.

Their work aims to develop rapid diagnostic tests, identify more effective treatments with fewer side effects, and explore biomarkers for predicting post-BMT complications. In addition, approved medications may be evaluated for faster, cost-effective treatment options.

> “Their work aims to develop rapid diagnostic tests, identify more effective treatments with fewer side effects, and explore biomarkers for predicting post-BMT complications.”

RECENT ACHIEVEMENTS AND PROGRESS

We are pleased to advise that the research team have made significant progress with the retrospective study, which is now complete and has been submitted for publication. Impressively, researchers analysed samples from 183 children and examined 78 different cytokines.

The research has so far found that two cytokines (CXCL9 and Chitinase 3 Like 1) measured before transplant could predict which children would develop post-transplant respiratory complications.

Both cytokines are linked to lung disease and suggest an overactive interferon-gamma pathway, meaning the immune system is reacting too strongly, which can lead to inflammation and damage in the lungs. Existing medications that block this pathway could potentially protect children from these complications.

The prospective study is also progressing well. The research team have recruited 20 participants and completed 12 months of follow-up and sample collection post-Hematopoietic Stem Cell Transplantation (HSCT). Recruitment is currently paused as we focus on analysing the collected biospecimens.

This includes flow cytometry, a technique used to analyse cells, and cytokines analysis, which measures proteins that play a key role in the immune response.

The team is now interpreting the generated data, and we look forward to sharing the results with you once they are available. Alongside both studies, the research team have recently published two papers.

One is an expert review, and the other examines the burden of pulmonary complications at RCH and Perth Children's Hospital.

Both papers include an acknowledgement of the generous support provided by The Bella Tripp Foundation. Copies of these impressive and valuable reports are included in the Appendix to this report.

> We are hopeful and confident that the prospective study will yield similarly helpful results, and once again this would not have been possible without the support of the Foundation.

KEY ACTIVITIES FOR 2025

Our key activities for 2025 are:

Analyse the data from the prospective study to identify any additional biomarkers worth exploring.

Attempt to validate the cytokines identified in the retrospective study in the prospective study.

Continue to apply for grants to fund the other ideas we have for using the samples collected, such as cell-free DNA and gene expression analyses.

THANK YOU FROM DR. SHIVANTHAN SHANTHIKUMAR

"On behalf of our research team, I would like to extend a heartfelt thanks for the support from The Bella Tripp Foundation.

Before we started this work, we had no way of predicting pulmonary complications, and now thanks to your funding, we have identified two potential biomarkers in the retrospective study which may be able to improve the care we deliver.

We are hopeful and confident that the prospective study will yield similarly helpful results, and once again this would not have been possible without the support of the Foundation.

As a team, we are so thankful that we can pursue this work so that we can reduce the burden and harm that comes from pulmonary complications of transplant."

Elevated plasma CXCL9 and CHI3L1 prior to HCT predict post-HCT pulmonary complications in children

Hannah Walker[1,3-4], Liam Grubbels[3], Diane Hanna[1,3-4], Theresa Cole[1,3-4], Gabrielle Haeusler[1,3-4], Shivanthan Shanthikumar[2-4] & Melanie Neeland[3-4]

1 Children's Cancer Centre, The Royal Children's Hospital Melbourne, Australia
2 Respiratory Medicine, The Royal Children's Hospital, Melbourne, Australia
3 Murdoch Children's Research Institute, Melbourne, Australia
4 The University of Melbourne, Australia

BACKGROUND & METHODS

Background:

- Pulmonary complications occur commonly post hematopoietic stem cell transplant (HCT) in children and contribute significantly to mortality.
- This mortality is influenced by the fact that pulmonary complications are often detected late, when irreversible lung damage has already occurred.
- Highlighting the importance to identify patients at risk of pulmonary as early as possible, prior to HCT.

Methods:

Plasma samples from 117 pre HCT patients and 66 healthy controls were used to measure 78 soluble immune analytes and clinical outcome data on post HCT complications was collected. (Fig 1). Principal components analyses (PCA) was used to determine the drivers of variability between groups. Mann-Whitney U tests were used to compare differences in pre-HCT plasma cytokine levels between groups. To explore biomarkers which predict post HCT lung disease, area under the curve (AUC) and receiver operating characteristic (ROC) curves were used.

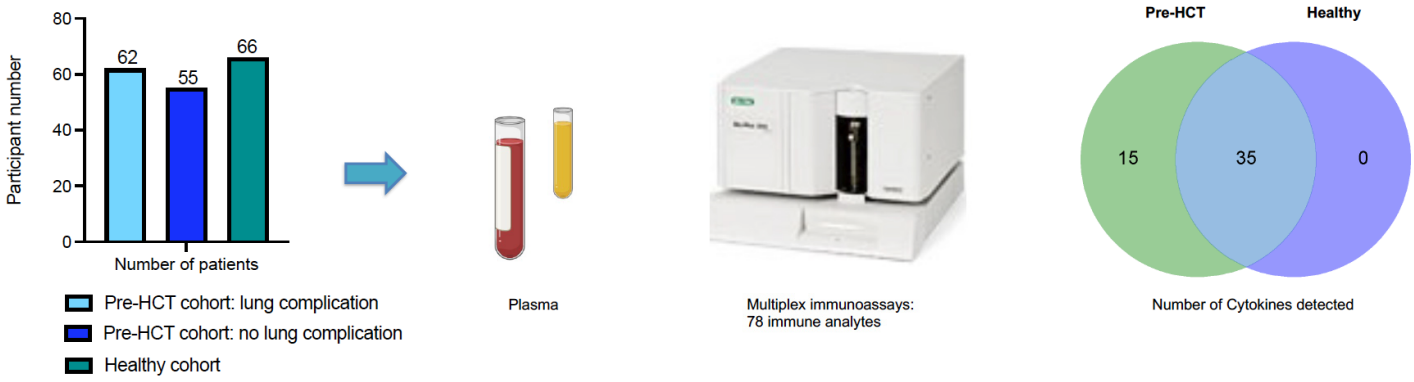


Figure 1. Study patients groups, methods and number of cytokines detected

Primary Objective:

1. To identify plasma biomarkers predictive of post HCT pulmonary complications in children undergoing HCT.

Secondary Objective:

1. To determine whether cytokine concentrations in normal children can be used as control patients for children undergoing HCT.

RESULTS - ADOLESCENTS WITH A MALIGNANT INDICATION PRE-HCT ARE PRO-INFLAMMATORY COMPARED TO HEALTHY PATIENTS

- Principal components analyses (PCA) of pre-HCT malignant (n=97) and healthy (n=66) patients plasma cytokine data showed clinical group (PC1, p=4.050E-32) and age (PC2; p=2.44E-03) contributed to the variability in cytokines seen (Fig 2).
- Comparing each age category of pre-HCT malignant and healthy patients showed clear differences across all age groups (Fig 2), with infants pre-HCT showing markedly reduced levels of 13 analytes compared to healthy infants.
- In contrast to what is seen in healthy children, pre-HCT patients became increasingly pro-inflammatory with increasing age when compared to healthy children. Adolescents showing upregulation of 10 analytes compared to healthy children.

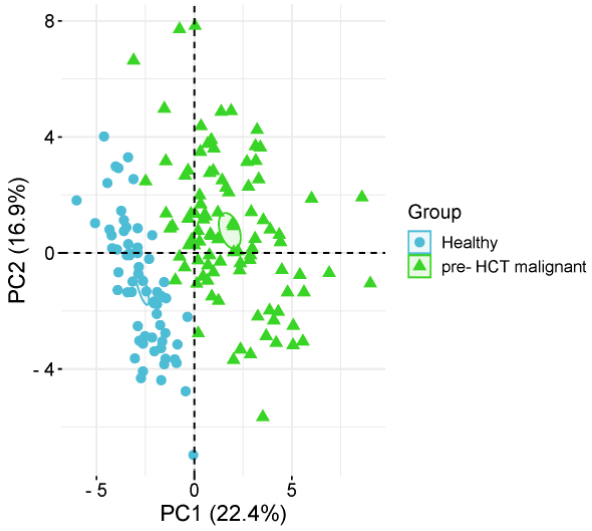
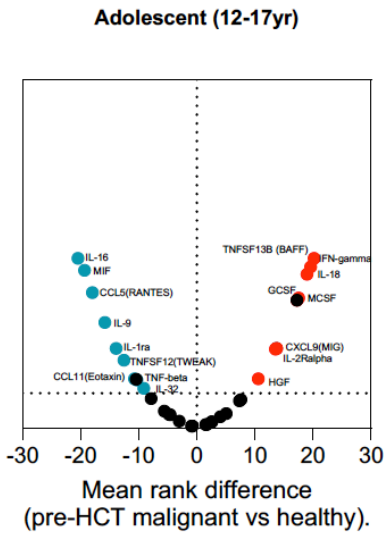
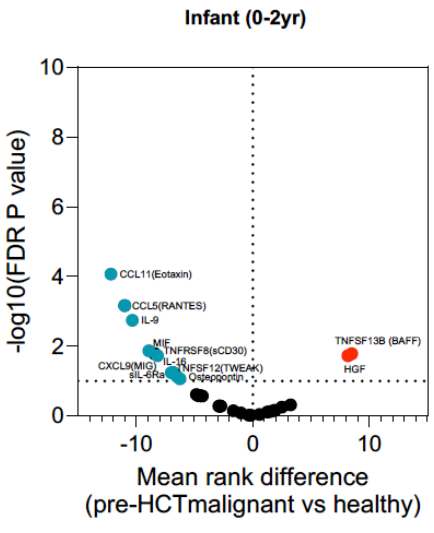


Figure 2: PCA analyses in pre-HCT malignant indication compared to healthy

Volcano plots of cytokines comparing infants) and adolescents , red represents cytokines that are upregulated and blue represents cytokines that were downregulated





Up in pre-HCT all ages

TNFSF13B (BAFF)

HGF

Down in pre-HCT all ages

CCL11 (Eotaxin)
CCL5 (RANTES)
TNF-β
IL-9
MIF
TNFSF12 (TWEAK)
IL-16
IL-1ra
CXCL10 (IP10)
Osteopontin
TNFRSF8 (sCD30)

RESULTS – CHARACTERISTICS OF LUNG COMPLICATIONS

- Pulmonary complications occurred in 53% (62/117) of transplant patients
- Demographics of these patients are shown in Table 1
- Malignant indication for HCT was most common in, 85% (53/62)
- The characteristics of these lung complications are shown in Figure 3, with early onset, non-severe, infectious lung disease being the most common type seen

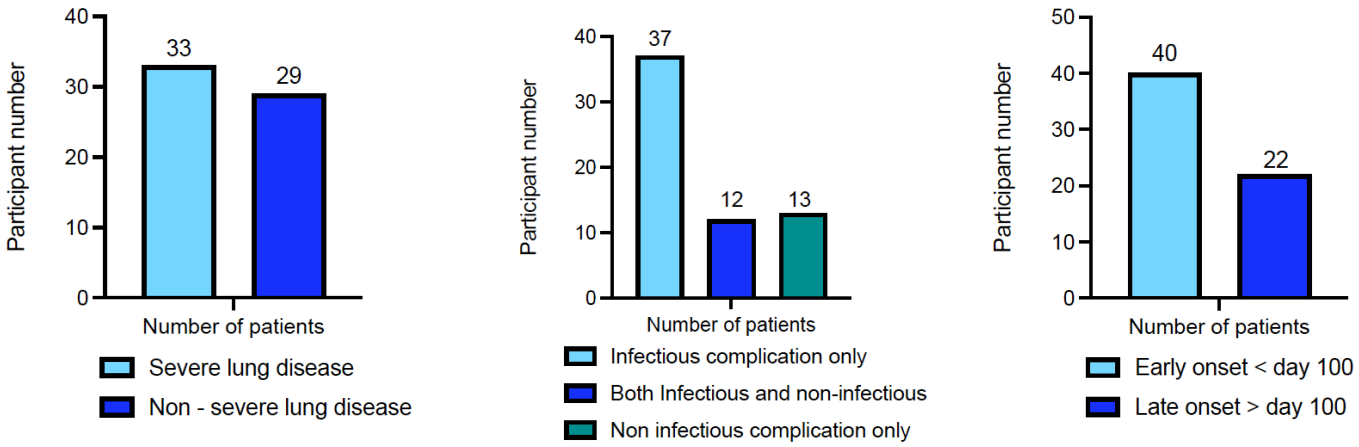


Figure 3: Characteristics of lung complications post HCT

Characteristic	Pulmonary complication N=62 (%)	No pulmonary complication N=55 (%)
Age (*)		
Median (yrs)	6	6
Male, n (%)	38 (61)	26 (47)
Diagnosis, n (%)		
Malignant	53 (85)	44 (80)
Non-malignant	9 (15)	11 (20)
Stem Cell Source, n (%)		
Bone marrow	15 (24)	25 (45)
Peripheral blood	38 (61)	29 (53)
Donor Type		
Haploidentical	13 (21)	12 (22)
Matched unrelated	32 (52)	18 (33)
Matched sibling	17 (27)	25 (45)
Conditioning Regimen, n (%)		
Myeloablative	56 (90)	47 (85)
GVHD Prophylaxis, n (%)		
MMF + CNI	14 (22)	15 (27)
TCR α/β depletion	6 (10)	6 (11)

TABLE 1: Patient characteristics undergoing HCT

*At time of blood sample within 3 months of day 0

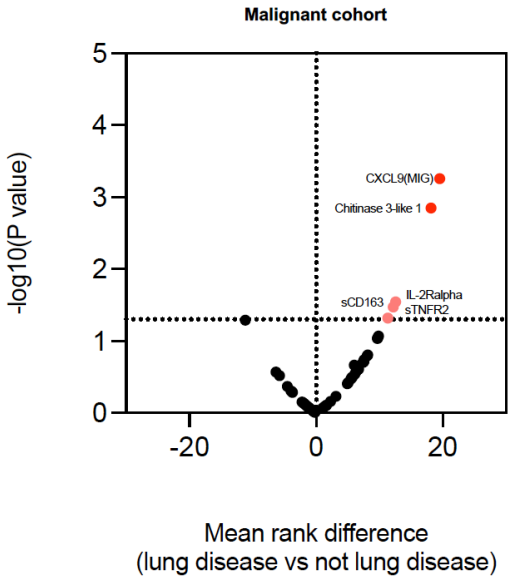
ALL, Acute lymphoblastic leukaemia, AML, Acute myeloid leukaemia, Bu, Busulfan, TREO, Treosulfan, TBI, Total Body irradiation, ATG, Antithymoglobulin, MTX, methotrexate, CNI, calcineurin inhibitor, MMF, mycophenylate, TCR, T cell receptor, HCT, Haematopoietic stem cell transplant

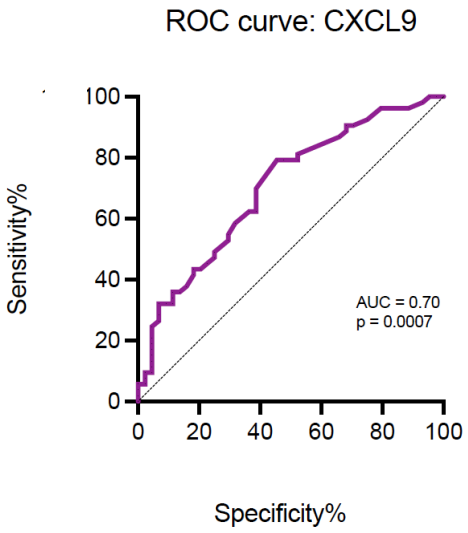
RESULTS – ELEVATED CXCL9 AND CHI3L1 PREDICT POST HCT PULMONARY COMPLICATIONS

- Elevated pre-HCT plasma CXCL9 (MIG) and Chitinase 3-like 1 (CHI3L1) were associated with post-HCT pulmonary complication outcomes (FDR p value p =0.0005 and p=0.0014 respectively) in patients with a malignant indication for transplant (Fig 4).
- AUC analyses showed that pre-HCT CXCL9 had significant predictive value (AUC=0.7, p=0.0007) and at a threshold > 7.075 log2 pg./mL (134.83 pg./mL) has a sensitivity of 69.81% and specificity of 61.36% (p=0.0007) to predict post-HCT pulmonary disease.
- Similarly, pre-HCT levels of CHI3L1 had significant predictive value (AUC =0.68, p=0.0016) and at a threshold > 11.15 log2 pg./mL (2272.39 pg./mL) has a sensitivity and specificity of 71.7% and 56.82%,respectively (p=0.013), to predict post-HCT pulmonary disease (Figure 4).

CONCLUSIONS

- Pre-HCT plasma levels of CXCL9 and Chitinase 3-like 1, both induced by IFN– γ, were associated with post-HCT pulmonary disease in children with a malignant indication for HCT.
- We also showed that healthy children are unlikely to be good controls for biomarker prediction in this cohort given the significant differences seen compared to age matched healthy controls
- A larger HCT cohort is therefore required to define the normal range of plasma cytokine levels for these patients as well as validate these biomarker findings.





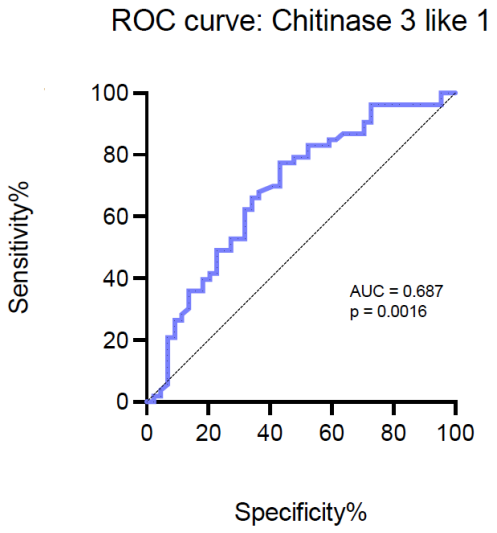


Figure 4. Volcano plot comparing patients who develop lung disease versus no lung disease (malignant indication); dark red shows cytokines that were upregulated and met the FDR threshold of < 0.1 and light red represent cytokines that met p value significance only , p< 0.05. ROC curve for both CXCL9 and CH3L1 also shown.

Events Highlights

CELEBRATING OUR FUNDRAISING

The Bella Tripp Foundation has been delighted to host a series of remarkable events, uniting communities, corporate partners, and supporters in our mission to improve the lives of children recovering from bone marrow transplants. These events have played a crucial role in raising essential funds and increasing awareness of respiratory complications following transplantation.

By fostering engagement and securing vital resources for research and advocacy, these gatherings have strengthened our commitment to driving meaningful change in post-transplant respiratory care.

THE BELLA TRIPP FOUNDATION INAUGURAL GALA EVENT

The Inaugural Gala Event was a landmark occasion for The Bella Tripp Foundation, signifying the beginning of an impactful journey towards improving health outcomes for children facing post-transplant respiratory complications. This extraordinary evening was a true celebration of generosity, unity, and collective commitment to change.

A special appreciation also goes to our valued partners and sponsors—Melbourne Storm, Betr, Maserati, Whitetop, Rutherford Entertainment, Simon West, Glasshaus, Prestige Events, Jacqui Felgate, Christian Lonzi, and Food and Desire—whose generosity and support helped create an unforgettable evening. Their contributions directly strengthen our ability to deliver memorable moments that leave lasting and positive impressions on our donors and community, in a way that grows awareness of our mission and purpose.

> “This extraordinary evening was a true celebration of generosity, unity, and collective commitment to change.”

At the Gala event, Yasmina Tripp, mother of Bella, shared an emotional tribute to her daughter: "Bella was my best friend, full of life and strength. The bravest person I have ever known."

In his speech at the event, Dr Shivanthan Shanthikumar underscored the critical importance of raising awareness and funding for research: "By investing in research and improving awareness, we have the opportunity to transform post-transplant respiratory care, ensuring better outcomes for children worldwide."

YARRAWONGA CHARITY GOLF DAY

We hosted a charity golf day in Yarrawonga, with Melbourne Storm players Grant Anderson and Young Tonumaipea joining supporters and sponsors.

Major sponsor Lotus Living played a key role in the event’s success, helping raise funds for ongoing research into respiratory complications following bone marrow transplants.

Beyond the sport and camaraderie, this event carried deep significance as Bella enjoyed many happy moments in Yarrawonga over many years with her family and friends.

Sincere thanks to Jen Severn and Lotus Living for their exceptional organisation and support of this golf day.

LUNCH AT SILVERWOODS RESORT

Set against the picturesque backdrop of Silverwoods Resort, 70 women gathered for a luncheon in support of The Bella Tripp Foundation. The event provided a valuable opportunity to share the Foundation's mission, strengthen community connections, and highlight the meaningful impact of our work. A portion of the proceeds was generously donated to the Foundation, directly supporting our ongoing programmes and initiatives. We are sincerely grateful to all who attended and contributed to the success of this special occasion.

CAULFIELD GRAMMAR SCHOOL FUNDRAISER

The Caulfield Grammar School Fundraiser was a testament to the dedication and generosity of students and educators in supporting The Bella Tripp Foundation. This student-led initiative successfully raised awareness and funds to advance research and treatment for post-transplant respiratory complications.

A special acknowledgment goes to Isabella Anderson, House Captain, whose leadership and commitment were instrumental in the event's success. Through meaningful discussions and proactive fundraising efforts, students showcased the power of youth-driven advocacy and community engagement. We extend our gratitude to everyone involved in making this initiative a success, demonstrating the profound impact collective action can have in driving change.

ROCK THE SQUIRES: A NIGHT OF MUSIC AND GIVING BACK

Rock the Squires was a resounding success, bringing together an incredible audience for an evening of live entertainment and philanthropy. Held on Saturday, 1st February 2025, at Squires Winery in Bundalong, the event featured Brendan Fevola and Ben Dixon as hosts, with a standout performance by Big and Horny, Melbourne's renowned 13-piece band.

> "Rock the Squires was a resounding success, bringing together an incredible audience for an evening of live entertainment and philanthropy."

With all proceeds supporting The Bella Tripp Foundation, the night was a powerful demonstration of how entertainment and community spirit can create meaningful impact. Attendees came together not only to enjoy the music but also to contribute to a cause that makes a real difference. We extend our sincere gratitude to everyone who attended, donated, and supported the event. Your generosity and enthusiasm helped make Rock the Squires an outstanding success.

HONOURING OUR SUPPORTERS

Each event we hold is a powerful reminder of the generosity, passion, and unwavering support of our community. Through shared moments of advocacy, fundraising, and awareness, we are making meaningful strides in improving post-transplant respiratory care for children. The dedication of our supporters has been the driving force behind our progress, allowing us to fund critical research, enhance medical treatments, and advocate for better care.

Every contribution—whether attending an event, making a donation, or simply spreading the word—plays a vital role in furthering our mission. Your involvement helps push groundbreaking research forward, amplifies the voices of families affected by post-transplant complications, and ensures that children receive the best possible care during recovery.

We are deeply grateful to everyone who has been part of this journey. Your support fuels our commitment to driving real change, and together, we can continue to make a lasting impact. We invite you to stay engaged, join us at future events, and help us build a world where no child faces post-transplant challenges alone.

To learn more about upcoming events and ways to contribute, please visit thebellatrippfoundation.org.au or follow us on social media.

“Your involvement helps push groundbreaking research forward, amplifies the voices of families affected by post-transplant complications, and ensures that children receive the best possible care during recovery.”

Looking Ahead: Our Future Goals

As we look to the future, our commitment to improving outcomes for individuals affected by transplant-related lung disease remains stronger than ever. The year ahead presents an opportunity to advance critical research, foster collaborations, and drive meaningful change.

By investing in early diagnostic tools, supporting innovative treatment development, and amplifying advocacy efforts, we aim to accelerate progress in this vital field. With the support of our community, partners, and donors, we are dedicated to making a lasting impact.

> “The year ahead presents an opportunity to advance critical research, foster collaborations, and drive meaningful change.”

Our key initiatives for the year include:

Advancing Research and Early Diagnosis

Expanding research funding to accelerate the development of early diagnostic tools, enabling timely intervention and improved patient outcomes.

Strengthening Medical Partnerships

Collaborating with leading medical institutions to drive clinical trials and pioneer innovative treatment options.

Elevating Fundraising Efforts

Launching impactful initiatives to secure increased financial support for groundbreaking research.

Amplifying Advocacy and Awareness

Expanding national and international advocacy efforts to ensure this critical issue receives the attention it deserves.

Acknowledgements and Heartfelt Thanks

ACKNOWLEDGING OUR SUPPORTERS

We extend our deepest gratitude to our donors, volunteers, and supporters. Many individuals and organisations have generously shared their knowledge, energy, expertise, and time in support of our mission.

Your generosity drives our mission, enabling us to fund vital research with the potential to save lives. Every single contribution—whether from a donor, a medical researcher, or an advocate—plays an essential role in our progress.

Specifically, we would like to acknowledge the people and businesses shown on the following pages for their invaluable contribution to the Foundation to date.

Please continue to support our purpose and visit thebellatrippfoundation.org.au and follow us @bellatrippfoundation on Instagram and Facebook.

Your engagement helps us raise awareness, expand our reach, and bring more people into our mission of changing lives. Together, we are paving the way for improved medical outcomes and a brighter future for children undergoing bone marrow transplants. Together, with your ongoing support, we aim to make a lasting impact.

> “Together, we are paving the way for improved medical outcomes and a brighter future for children undergoing bone marrow transplants. Together, with your ongoing support, we aim to make a lasting impact.”

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ORIGINAL ARTICLE

Pulmonary complications post allogeneic haematopoietic stem cell transplant in children

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Abstract

Objectives. Haematopoietic stem cell transplant (HCT) is a cellular therapy that, whilst curative for a child's underlying disease, carries significant risk of mortality, including because of pulmonary complications. The aims of this study were to describe the burden of pulmonary complications post-HCT in a cohort of Australian children and identify risk factors for the development of these complications. **Methods.** Patients were identified from the HCT databases at two paediatric transplant centres in Australia. Medical records were reviewed, and demographics, HCT characteristics and pulmonary complications documented. Relative risk ratio was used to identify risk factors for developing pulmonary complications prior to first transplant episode, and survival analysis performed to determine hazard ratio. **Results.** In total, 243 children underwent transplant during the study period, and pulmonary complications occurred in 48% (117/243) of children. Infectious complications were more common (55%) than non-infective complications (18%) and 26% of patients developed both. Risk factors for the development of pulmonary complications included the following: diagnoses of MPAL (RR 2.16, $P = 0.02$), matched unrelated donor (RR1.34, $P = 0.03$), peripheral blood (RR 1.36, $P = 0.028$) or cord blood (RR 1.73, $P = 0.012$) as the stem cell source and pre-existing lung disease (RR1.72, $P < 0.0001$). Children with a post-HCT lung complication had a significantly increased risk of mortality compared with those who did not (HR 3.9, $P < 0.0001$). **Conclusion.** This study

demonstrates pulmonary complications continue to occur frequently in children post-HCT and contribute significantly to mortality. Highlighting the need for improved strategies to identify patients at risk pre-transplant and enhanced treatments for those who develop lung disease.

Keywords: haematopoietic stem cell transplant, infection, paediatric, pulmonary complications

INTRODUCTION

Allogeneic haematopoietic stem cell transplant (HCT) is a cellular therapy used to treat children with a range of life-threatening conditions, including inborn errors of immunity, metabolic storage disorders, haemoglobinopathies and haematological malignancies. Whilst the goal of HCT is to cure the child's underlying condition, it has a range of potentially serious complications, including pulmonary complications.[1] Pulmonary complications following HCT contribute a high proportion of non-relapse mortality.[2–7] Because of advances in supportive care, antimicrobial prophylaxis and treatments, outcomes from infectious pulmonary complications have improved in recent years.[8] In comparison, non-infectious pulmonary complications including bronchiolitis obliterans (BO), idiopathic pneumonia syndrome (IPS) and engraftment syndrome[1,5] have limited methods for early detection and targeted therapy, with outcomes remaining poor.[9] Large, primarily adult, studies have identified a range of risk factors for the development of post-HCT pulmonary complications. These include chemotherapy agents such as busulfan, cyclophosphamide and methotrexate, which are frequently used as part of the patients conditioning regimen.[8,10,11] Other risk factors include total body irradiation (TBI), pre-existing lung disease,[8] HLA mismatch, graft vs host disease (GvHD) and clinical indication for HCT.[2,8,12]

There are limited published data on the specific types of pulmonary complications in children post-HCT and their long-term outcomes.[8] Research has previously focussed on the experience in adults post-transplant or combined adult and paediatric cohorts. Rates of pulmonary complications post-HCT in adults are reported to occur between 30% and 40%.[13,14] Published data in paediatric patients, collected between 1996 and 2015,[2–4,7] reported incidence of pulmonary complications (36–74%) and associated mortality (25–65%) and have varied widely. Importantly, there have been several significant changes in practice since these data were generated, including increased use of immunotherapy prior to HCT, changes in chemotherapy conditioning regimens for acute lymphoblastic leukaemia (ALL) with improved outcomes for a TBI backbone (FORUM trial),[15] increased use of reduced intensity conditioning (RIC) regimens and an increase in the use of haploidentical donors.[16] There have also been advancements in supportive care post-HCT, including pre-emptive screening and treatment for viral reactivation and increased use of steroid sparing agents for GVHD post-HCT. These factors, which have changed the landscape of paediatric allogeneic transplant in the last 10 years, may have impacted the characteristics and impact of pulmonary complications post-HCT. Another key limitation of previous studies is that they have combined outcomes for both autologous and allogeneic transplants, which are likely to have different risk profiles for post-HCT complications. Additionally, many of these studies were conducted in single centres and may not consider variability between transplant centres.

The primary aim of this study was to describe the characteristics and impact of pulmonary complications post-HCT in a multicentre paediatric cohort undergoing allogeneic HCT, reflecting current practice. The secondary aims of this study were to identify both protective and risk factors for the development of pulmonary complications and to identify factors predicting poor outcome following pulmonary complications.

RESULTS

Cohort demographics and aetiology of pulmonary complications

The study identified 268 episodes of paediatric allogeneic HCT in 243 individual patients (Figure 1a). The median follow-up was 32.5 months post HSCT. Pulmonary complications

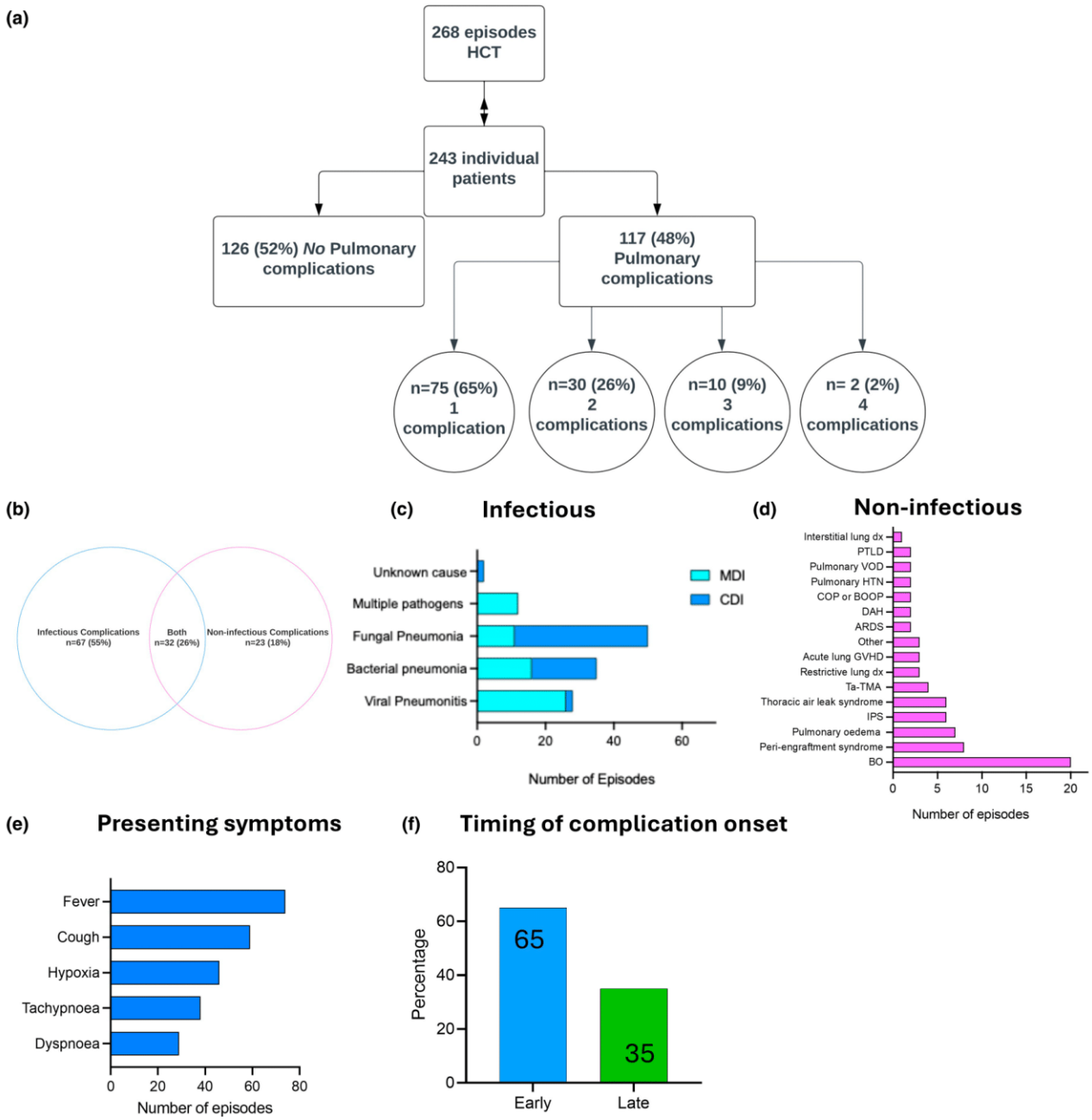

Figure 1. Cohort demographics and aetiology of pulmonary complications **(a)** Flow chart depicting patient and transplant episode and rates of development of pulmonary complications **(b)** Aetiology of lung complications shows infectious complications were the most common cause **(c)** Infectious complications aetiology including MDI and CDI: shows high proportion of possible fungal infection, viral pneumonitis and bacterial pneumonia. **(d)** Types of non-infectious pulmonary complications: shows bronchiolitis obliterans (BO) and peri-engraftment syndrome were the most common causes. **(e)** Presenting symptoms of pulmonary complication: shows non-specific symptom of fever was the most common. **(f)** Timing of complication onset: demonstrating most complications started early post-transplant in the first 100 days.

occurred in 48% (117/243) of patients, and within this group, 35% (42/117) of patients developed more than one pulmonary complication (Figure 1a). Demographics of the cohort including age and indication for *first transplant episode* (pulmonary complication; $n = 115$ patients) are shown in Table 1. The most common indications for HCT were malignancies, B-cell acute

Table 1. Demographics and risk of developing pulmonary complication *post first* HCT episode

Characteristic	Pulmonary complication (*n* = 115)	No pulmonary complication (*n* = 128)
Age in years at HCT Day 0		
Median (IQR)	6.8 (2.3–13.8)	7.05 (3.67–11.6)
Age < 1 year (*n*, %)	19 (16.5)	15 (11.8)
Age 1.1–4 years	18 (15.6)	24 (18.9)
Age 4.1–12	42 (26.5)	61 (47.6)
Age > 12.1 years	37 (32.1)	27 (21.2)
Male, *n* (%)	73 (51.4)	69 (48.59)
Diagnosis, *n* (%)		
Malignant	75 (48.4)	80 (51.6)
Non-malignant	54 (50.4)	53 (49.5)
Specific diagnoses		
B-ALL	19 (16.5)	25 (19.5)
AML	21 (18.2)	25 (19.5)
Mixed phenotype ALL	5 (4.3)	0
Primary immunodeficiency (any)	28 (23.9)	23 (17.9)
Haemoglobinopathies	4 (3.5)	3 (2.3)
Metabolic disorder	8 (7)	3 (2.3)
Aplastic anaemia	3 (2.6)	13 (10.1)
Stem cell source, *n* (%)		
Bone marrow	34 (29)	67 (52)
Peripheral blood stem cells	68 (59)	57 (44)
Cord blood	14 (12)	4 (0.03)
Donor type		
Haploidentical	28 (24)	30 (23)
Matched unrelated	58 (50)	47 (37)
Matched sibling	29 (25)	51 (40)
Conditioning regimen		
Myeloablative	85 (73)	80 (62.5)
Conditioning details		
BU/FLU	42 (36.5)	50 (39)
TREO/FLU	32 (27.8)	25 (19.5)
CY	17 (14.8)	32 (25)
MEL	8 (7)	13 (10.1)
TBI any, *n* (%)	23 (20)	30 (23.4)
Serotherapy		
Alemtuzumab	18 (15.6)	20 (15.6)
ATG	61 (53)	54 (47)
GVHD prophylaxis		
MTX + CNI	25 (21.7)	29 (12)
MMF + CNI	50 (43.5)	47 (36.7)
CNI only	31 (27)	37 (29)
Methylprednisolone	4 (3.5)	0
TCR α/β depletion	17/68 (25)	17/57 (29)
Required a second HCT	18 (15)	11 (8.6)
Previous B-cell immunotherapy[a]	14 (12.2)	20 (15.6)
Chemotherapy pre-HCT	64 (55.7)	80 (62.5)
Radiation (any including TBI)	3 (2.6)	10 (7.8)
Abnormal chest CT pre-HCT[b]	72 (69.2)	59 (45)
Infection history pre-HCT		
Active lung infection 30 days pre-HCT	21 (18.2)	13 (10.2)
Lung disease history pre-HCT[c]	63 (54.7)	36 (28.2)
Pneumonia	31 (27)	17 (35.4)
Bronchiectasis	6 (5.2)	1 (0.8)
Mechanical ventilation	10 (8.7)	5 (3.9)

AML, Acute Myeloid Leukaemia; ATG, Anti-thymocyte globulin; B-ALL, B-Cell Acute Lymphoblastic Leukaemia; BU/FLU, Busulfan/Fludarabine; CNI, Calcineurin inhibitor; CY, Cyclophosphamide; IQR, Interquartile range; MEL; Melphalan; MMF, Mycophenylate; MTX, Methotrexate; TBI, Total Body Irradiation; TCR α/β, T-Cell receptor alpha/beta; TREO/FLU, Treosulfan/Fludarabine.

[a]Previous Immunotherapy included blinatumomab, inotuzumab and CAR-T therapy.

[b]Abnormal CT included nodules, pleural effusion and consolidation.

[c]Lung dx included any of the following: asthma, pneumonia, bronchiectasis, ARDS, ACS, and mechanical ventilation.

lymphoblastic leukaemia (B-ALL) [18% (44/243)] and acute myeloid leukaemia (AML) [19% (46/243)]. Transplant occurred at a median age of 7 years (IQR 3.1–12.75).

The aetiology of the pulmonary complications is detailed in Figure 1b–d. There were 99 infective diagnoses and 55 non-infective pulmonary complication episodes identified in patients post-HCT (follow-up included if patients developed a lung complication post second transplant if captured in the study period). Many patients received more than one diagnoses in both categories, reflected in the total diagnoses exceeding the number of patients. In patients who developed an infective complication, there were 63 episodes of MDI and 37 episodes of CDI. The contribution of types of MDI is shown in Figure 1c; viral pneumonitis was the most common in 11% (26/243) followed by bacterial pneumonia, which is common in 7% (16/243). MDI related to fungal infection included both probable and proven fungal infection in 4.5% (11/243). Infection with multiple organisms, most commonly viral and fungal infection, was

reported in 5% (12/243). The most common respiratory viruses detected on PCR were cytomegalovirus (CMV) [5% (13/243)], rhinovirus [4.5% (11/243)], adenovirus [3% (8/243)] and human parainfluenza types 1–3 [4% (10/243)] (Supplementary table 2). Organisms identified on BAL sampling are shown in Supplementary table 2 and included two episodes of *Pseudomonas* spp.

Episodes of clinically defined respiratory tract infection are shown in Supplementary table 3; the most commonly occurring were IFI in 16% (39/243); bacterial pneumonia in 8% (19/243) of episodes; and viral pneumonitis in 1% (2/243). Of the CDI related to possible fungal disease, 64% (25/39) were also attributed to another microbiological, clinical or non-infectious pulmonary cause. This reflects the clinical strategy to treat pre-emptively for possible IFI in patients post-HCT with suspicion of a pulmonary complication whilst awaiting further diagnostics. There was one episode of sepsis, which was presumed related to a pulmonary source but was culture negative, and one episode of laryngotracheobronchitis, which was also reported as a CDI.

Overall, there were 54 episodes of pulmonary IFI [22%, (54/243)], the majority were classified as possible fungal infection only [72%, (39/54)]. There was low frequency of both probable 13% (7/54) and proven pulmonary 7% IFI (4/54). Of the proven and probable, three were attributed to *Aspergillus* spp., two because of *candida* spp. and two because of PJP. Both patients with episodes of PJP were identified to have poor compliance with the use of PJP prophylaxis medications (trimethoprim and sulfamethoxazole).

The non-infective diagnoses are shown in Figure 1d; the most commonly occurring were BO [8%, (20/243)], engraftment or peri-engraftment syndrome [3%, (8/243)], idiopathic pneumonia syndrome (IPS) [2%, (6/243)] and thoracic air leak syndrome [2%, (6/243)]. Of note, some individual patients in this group also developed more than one non-infective diagnoses, including one patient developed pulmonary haemorrhage, which led to progressive pulmonary fibrosis and thoracic air leak syndrome.

Figure 1e shows the most common presenting symptoms and signs at the onset of the pulmonary complication episode ($n = 125$). These included fever [59%, (74/125)], cough [47%, (59/125)] and hypoxia [37%, (46/125)]. Pulmonary complication episodes occurred initially more frequently in the first 100 days of HCT ($n = 81$, 65%) compared with after 100 days ($n = 44$, 35%) (Figure 1f). The most common initial imaging performed was a chest x-ray [72%, (90/125)], followed by CT scan [57%, (71/125)]. Of those who had a CXR as an initial investigation, 35% (32/90) had a subsequent CT chest. Bronchoalveolar lavage was performed in 45% (56/125) and lung biopsy in only 6% (8/125).

Infection prophylaxis most prescribed for all patients at the time of hospital admission was the combination of trimethoprim and sulfamethoxazole for PJP [89%, (216/243)], acyclovir for viral prophylaxis [95%, (232/243)] and fluconazole [72%, (174/243)] or micafungin [16%, (39/243%)] for fungal prophylaxis. Data on initial treatment were also collected in the first 24 h post onset of pulmonary complication symptoms. A large proportion of patients were already receiving antibiotics at the time of presentation [43%, (54/125)], with piperacillin–tazobactam the most common, in keeping with empiric treatment of febrile neutropenia in this group. Piperacillin–tazobactam was also the most frequently prescribed initial antibiotic for treatment in this group [22%, (28/125)]. Liposomal amphotericin [19%, (24/125)] was the most common initial antifungal treatment prescribed. Treatment for specific non-infective pulmonary complications is detailed in Supplementary table 4, with corticosteroids (both inhaled and/or systemic) the most prescribed agent [27%, (34/125)].

Mortality associated with the development of pulmonary complications and implications of disease severity

Survival analysis revealed a 3.9-fold increase in mortality in patients who developed a pulmonary complication compared with those who did not (Figure 2a, $P < 0.0001$). Within the cohort who developed pulmonary complications, admission to a paediatric intensive care unit (PICU) was associated with an 11.4-fold increase in mortality compared with no PICU admission ($P < 0.0001$, Figure 2b). The requirement for ventilation related to the pulmonary complication was associated with a 70-fold increase in mortality compared with no requirement for ventilation ($P < 0.0001$, Figure 2c).

Supplementary table 5 details aspects of disease severity in the total group and those who developed pulmonary complications. Among

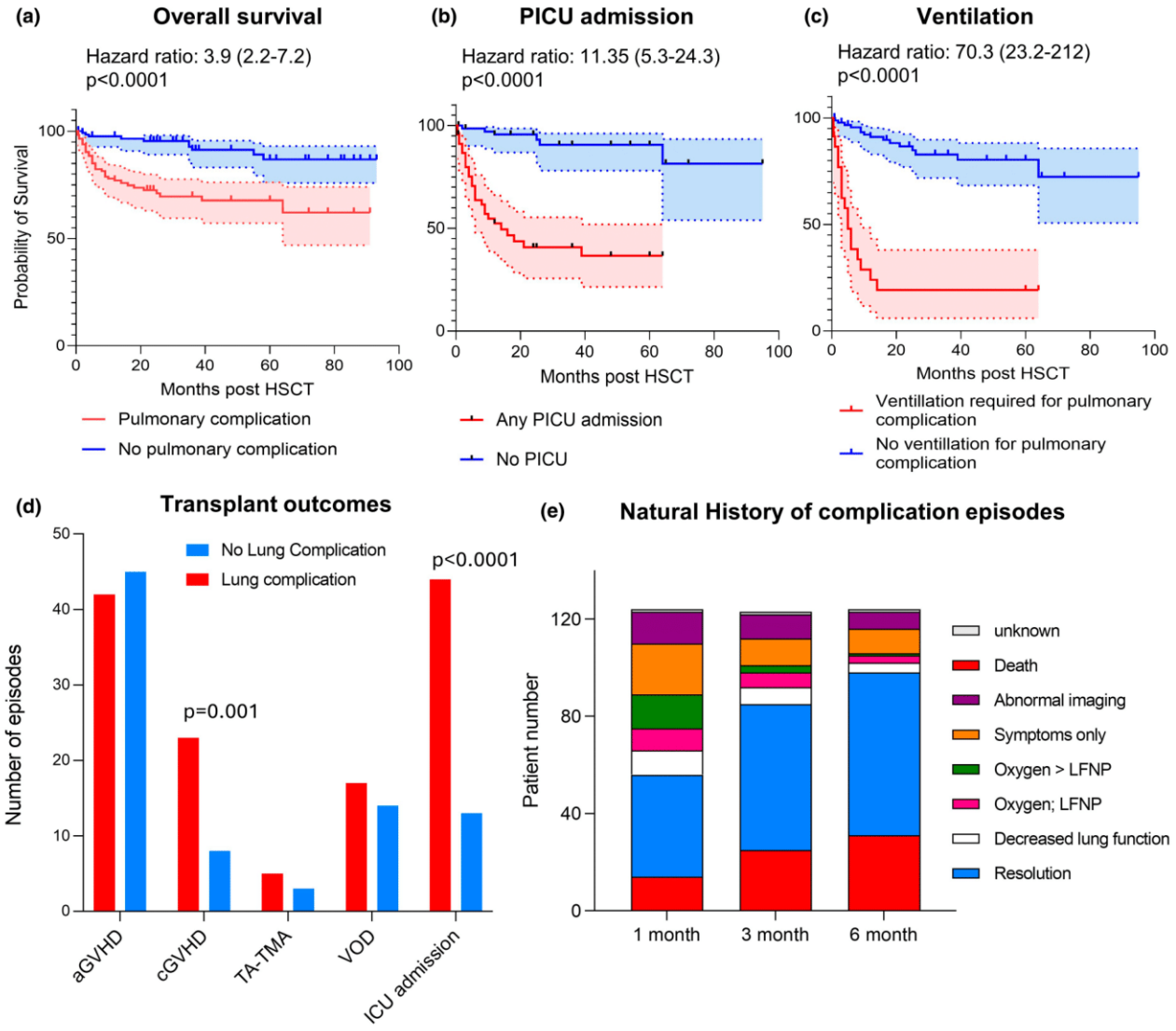

Figure 2. Mortality in children who developed pulmonary complications and general transplant outcomes. **(a)** Worse overall survival of children who developed a pulmonary complication post-HCT: survival analyses performed using Kaplan–Meyer method, HR 3.9, $P < 0.0001$. **(b)** Children who required PICU admission for pulmonary complication; inferior survival, HR 11.35 $P < 0.0001$. **(c)** Ventilation in children who developed a pulmonary complication had the poorest survival: Compared to children with a lung complication who did not require ventilation, HR 70.3 $P < 0.0001$. **(d)** General transplant outcomes in children who developed a pulmonary complication: increased rates of cGVHD $P = 0.001$ and any ICU admission $P < 0.0001$. **(e)** Natural history of children who developed a pulmonary complication: The majority of children have complete resolution of disease without residual lung imaging changes or symptoms at 6 months post disease onset.

those with pulmonary complications, 26% (30/115) required admission to a paediatric intensive care unit, and 19% (22/115) developed chronic pulmonary disease. Mortality associated with pulmonary disease was 6% (14/243), comprising the majority of non-relapse mortality (NRM). This was higher than the number of patients whose mortality was attributed to relapse after the *first* transplant in the total cohort (4.5%, 11/243). In the cohort requiring a *second* transplant ($n = 29$), mortality because of disease recurrence was more common [28%, (8/29)] than death from pulmonary disease (14%, 4/29).

A comparison of general transplant outcomes between the group who developed pulmonary complications and those who did not showed

higher rates of chronic graft versus host disease (cGVHD) [20%, (23/115) vs 6%, (8/128), $P = 0.0018$] and admission to PICU for any reason [38%, (44/115) vs 10%, (13/128), $P < 0.0001$] in those who developed pulmonary disease (Figure 2d). There were no statistical differences in rates of acute graft versus host disease (aGVHD) [37%, (42/115) vs 36%, (45/125), $P = 0.89$], VOD [15%, (17/115) vs 11%, (14/128), $P = 0.37$] or transplant-associated thrombotic microangiopathy [4%, (5/115) vs 2%, (3/128), $P = 0.48$] between the groups (Supplementary table 6).

The natural history of patients who developed a pulmonary complication episode is shown in the response to therapy at 1, 3 and 6 months post presentation of the pulmonary complication (Figure 2e). Overall, 58% (67/115) had complete resolution by 6 months, 3% (4/115) had ongoing oxygen requirement, and 26% (30/115) had died.

Risk factors for the development of post-HCT pulmonary complications

We identified several risk factors associated with the development of post-HCT pulmonary complications (Figure 3 and Table 1). This included diagnoses of mixed phenotype ALL (RR 2.16, $P = 0.02$), matched unrelated donor (RR 1.34, $P = 0.03$), peripheral blood (RR 1.36, $P = 0.028$) or cord blood (RR 1.73, $P = 0.012$) as the stem cell source, and pre-existing pulmonary disease (RR 1.72, $P < 0.0001$) including abnormal CT pre-HCT (RR 1.42, $P = 0.02$). The following protective factors were associated with a decreased risk of developing post-HCT pulmonary disease: a diagnoses of aplastic anaemia (RR 0.38, $P = 0.02$), bone marrow as the stem cell source (RR 0.59, $P = 0.0003$) and having a matched sibling as the available donor (RR 0.68, $P = 0.02$). There was no statistically significant difference between patients who received a particular chemotherapy agent or TBI as part of the conditioning regimen (Figure 3).

Non-relapse mortality because of pulmonary disease

The detail of 18 patients who died because of a pulmonary complication, post first or second HCT, is included in Supplementary table 7. The following themes were identified in this high-risk group including the following: higher incidence of MMRD 54.5% (12/22) and PBSC 77.3% (17/22) compared with the whole cohort. Patients were also more likely to present at the time of complication with hypoxia 75% (15/20) compared with cough 30% (6/20) and fever 40% (8/20) in the cohort who died. As Supplementary table 7 illustrates patients were also more likely to have multiple aetiological factors, both infective and non-infective in 77%, (14/18) compared with the whole cohort in 26% (32). CMV disease was also a frequent contributor to aetiology of lung disease in these cases of TRM in 44% (8/18) and the most commonly implicated pathogen. There was no statistical difference between TRM in the patients who had early onset compared with late onset of the pulmonary complication $P = 0.784$, RR 0.8 (CI 0.36–1.89).

DISCUSSION

This study highlights that pulmonary complications occurred frequently post allogeneic HCT and were associated with high NRM in children. The survival data in this group clearly show the negative impact of pulmonary complications on survival of children post-HCT. Particularly, that death related to pulmonary complications occurred more frequently than malignant disease relapse in the setting of first transplant. The interesting dichotomy of this study showed that survivors post an episode of pulmonary complication were more likely to have minimal morbidity and recover completely within 6 months. Alternatively, the next most common outcome was death because of pulmonary complication, and this was significantly more likely if the patient needed ventilation support in PICU. Similar findings related to incidence and impact have been reported previously,[2,3,7] describing rates of pulmonary complication and mortality from pulmonary complications between 25–36% and 24–65%, respectively. The detail related to survivors in this study has not been detailed in previous studies. Similar risk of mortality related to the need for PICU admission (because of all causes) in children post-HCT was reported in a recently published large multicentre study by Zinter and colleagues, which showed survival was reduced to 52.5% at 1 year in those who required a PICU admission.[17]

The risk factor analysis in this study highlighted several factors related to the patient pre-transplant, including pre-existing pulmonary disease and the presence of an abnormal chest CT scan associated with a higher risk of developing

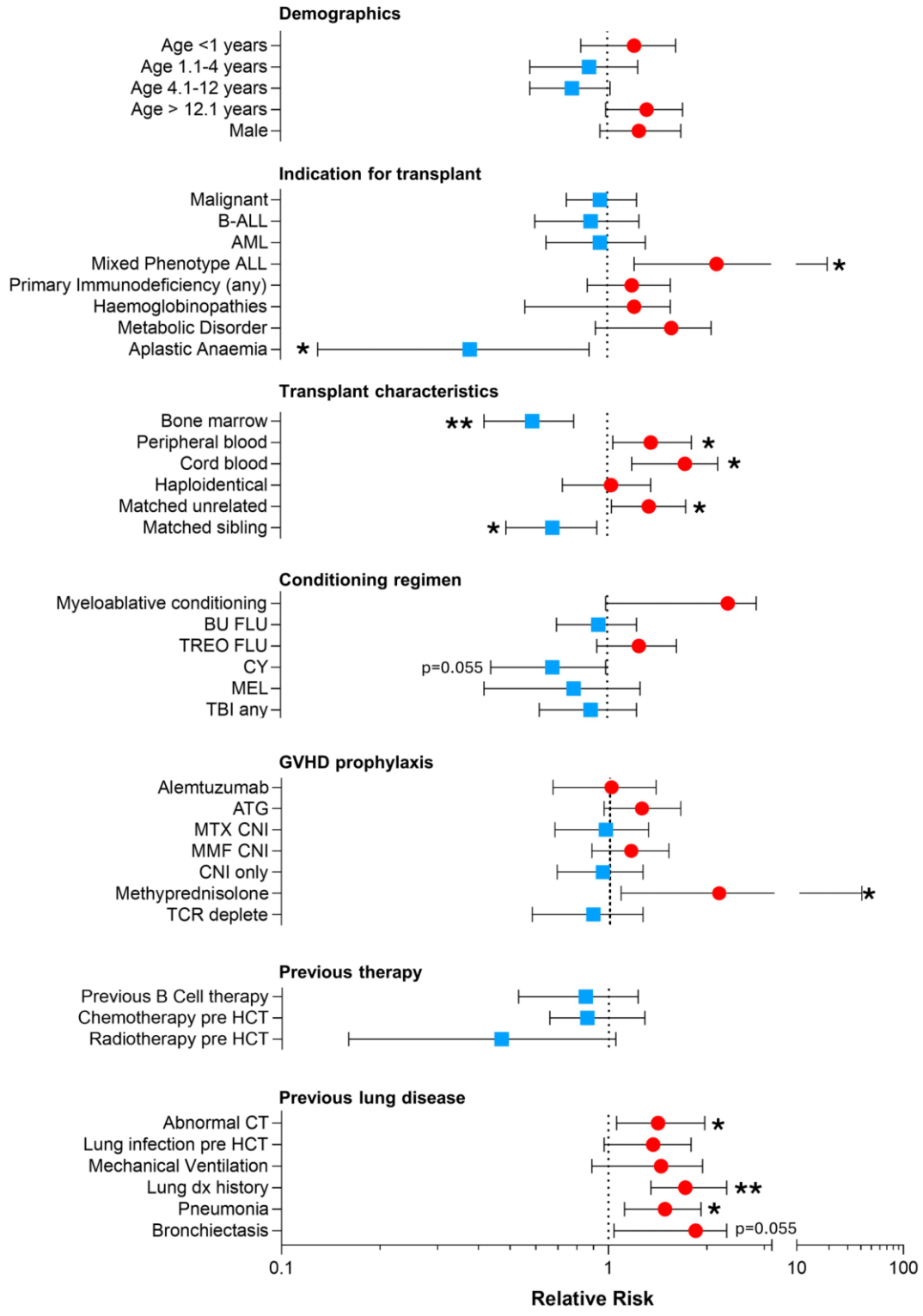

Figure 3. Risk factors for the development of post-HCT pulmonary complications. Forrest plot demonstrating risk factors and protective factors pre transplant and related to indication for transplant, previous lung disease, donor characteristics, conditioning regimen and GVHD prophylaxis. Relative risk with 95% confidence interval shown, $*P < 0.05$ and $**P < 0.01$.

pulmonary disease post-HCT. This demonstrates the importance of understanding a child's exposure to specific pulmonary disease and the importance of a pre-HCT baseline chest CT scan, recently endorsed as a recommendation in an updated American Thoracic Society clinical practice guideline related to paediatric BOS.[18] Both can be performed in most children pre-HCT, compared with lung function pre-HCT, which is limited to children old enough and well enough to perform these tests. Consideration of pre-HCT consultation with a paediatric respiratory physician may also be beneficial in this high-risk group of patients. Patients who received a transplant that was from a matched sibling donor using bone marrow as the source had the lowest risk of developing post-transplant pulmonary disease, and this is reflective of the low NRM reported in this group. Unfortunately, the majority of patients do not have a matched sibling donor available, and further research is required to identify why having a MUD is related to increased risk of pulmonary complications. Another factor associated with a decreased risk of developing post-HCT pulmonary disease was a diagnoses of aplastic anaemia. This is likely related to the reduced intensity conditioning regimen, use of bone marrow as a cell source and matched sibling as a donor preference (compared with a trial of immunotherapy).

Interestingly, in this cohort, there was no difference in rates of acute GVHD between the group who developed post-HCT pulmonary disease compared with those who do not. In contrast, cGVHD rates were higher in the group who developed post-HCT pulmonary disease, which has been shown in an earlier study.[7] It is important to highlight cGVHD often occurred after the onset of pulmonary complications had become clinically apparent and included some patients who received a diagnoses of BO, a type of cGVHD. This finding suggests there may be donor versus host interactions early in transplant whose aetiology overlaps with both the development of cGVHD and the development of pulmonary complications.

Several risk factors previously reported to increase a patient's risk of post-HCT pulmonary disease related to specific conditioning agents in adults[12] including TBI (high or low dose) were not statistically significant in this cohort. This may have been because of relatively small sample size and variety of conditioning regimens used, with multiple combinations of chemotherapy ± radiation. A recent systematic review looked at pulmonary complications post-TBI in children and identified six studies that reported on non-infectious pulmonary complications, particularly IPS in this group.[19] This review[19] also did not identify an association between TBI and IPS as a single factor but highlighted that multiple variables may have contributed to this finding including the following: systemic chemotherapy agents that carry different risk profiles, limitations on reporting of lung point dose, variations in dose rate and contribution from graft- vs host-related factors. Compared with previously published single-centre retrospective studies,[2–4,7] this study was conducted in a comparatively large population size, was performed across more than one centre, included only children who underwent allogeneic transplant (compared with both autologous and allogeneic) and is the first described for the Australian cohort. Overall, the risk factor analysis highlights that no single factor causes pulmonary disease in isolation, but the culmination of factors pre- and post-HCT.

This study's limitations include its retrospective nature and potential underreporting of less severe pulmonary complications managed outside the tertiary centre. Data from patients aged 18 or older who transitioned to adult care or were treated interstate may have also been missed. The classification of pulmonary complications was subject to investigator bias; however, all positive microbiological pulmonary investigations were collected, and diagnoses were classified as infective, non-infective or both using the definitions described in Supplementary table 1. The sample size was insufficient for multivariate analysis of risk factors, an area for future research requiring a substantial increase in patient numbers for meaningful significance.

The risk factor analysis performed in this study identifies potential preventative and therapeutic strategies for managing children at risk of post-HCT pulmonary complications. Prior to HCT, strategies could include early multidisciplinary involvement of at-risk patients, such as involvement by infectious disease, respiratory and transplant teams to establish a pre-emptive plan for therapy should the patient develop a post-HCT pulmonary complication. The role of pre- and post-transplant chest physiotherapy is also an area that requires consideration of implementing in children old enough to participate. A recently published study in adults showed a significant improvement in lung function in those who were randomised to receive chest physiotherapy in the

3 weeks prior to HCT admission.[20] Whether this translates to children and leads to a reduction in post-transplant pulmonary complications requires further research but is an appealing and low-risk strategy that could be incorporated into clinical care.

Education for patients, families and clinical staff who care for these patients on the subtle early signs and symptoms of these pulmonary complications is also an important strategy to improve awareness. Prevention of infectious complications post-HCT involves a combination of pre-emptive screening and pharmacological methods, including antiviral prophylaxis and antifungal prophylaxis. In this study, the most used antifungal agents, fluconazole or micafungin, have inferior mould cover in comparison with posaconazole and voriconazole. In patients with an increased risk of post-HCT pulmonary complications such as an abnormal CT chest or who have had previous lung disease, it is possible they may benefit from antifungal prophylaxis with broader mould coverage. This requires further prospective studies and the interactions with CNI require dose adjustment considerations in the setting of azole antifungal use.

Because of the prevalence of CMV in the patients who died because of pulmonary disease, post-HCT prophylaxis strategies for this cohort to reduce severe CMV disease are required. There is established evidence for the use of letermovir prophylaxis in CMV seropositive patients for the reduction in post-HCT mortality.[21] This is increasing in paediatric populations, and some centres have adopted the use of letermovir in high-risk groups (recipient +/donor −) based on safety and efficacy of its use off label.[22] Letermovir is not currently approved in Australia for children but is available through direct access schemes.

In terms of improved treatment strategies, more research is required into the biological drivers of paediatric non-infectious pulmonary complications, to identify targeted strategies that minimise the need for prolonged high-dose steroids. A current example is the use of etanercept, a TNFα inhibitor, used as a treatment in children with IPS, because of the identification of elevated TNFα levels in BAL and plasma of patients with this complication.[23] Aside from IPS and Ta-TMA (target agent eculizumab), there is limited evidence for survival benefit of targeted therapies for this group of non-infectious pulmonary complications. Certain pulmonary complications post-HCT, such as DAH with dismal survival, are at most urgent need of improved therapeutic strategies.[24]

Pulmonary complications as a group of disorders are heterogenous and encompass a variety of different diagnoses. This study shows that a significant proportion of paediatric patients experienced more than one pulmonary complication and that the aetiology of the pulmonary disease is complex. This highlights the challenge for clinicians caring for these patients and that there are factors downstream that continue to influence patient outcomes. For example, studies have shown the presence of a virus in the respiratory tract pre-HCT (e.g. rhinovirus) may 'prime' the pulmonary system to be more at risk of being a target for post-transplant graft versus host interactions and non-infectious pulmonary diseases such as IPS.[25] This relationship may be bidirectional, for example treatment of many non-infectious pulmonary diseases utilised corticosteroids, which may increase the risk of developing and/or worsening infective complications in these patients. Rather than defining a patient as having infectious or non-infectious pulmonary disease, a more meaningful approach may be to consider the many factors (both microbiological and transplant-related) that lead to pulmonary disease manifestation. This study shows that pulmonary complications remain an important cause of morbidity and mortality in children post-HCT, and clinicians need to have a high index of suspicion for their development in this population.

METHODS

Cohort description and clinical definitions

This was a retrospective study of children (age < 18 years) who underwent allogeneic HCT between 2016 and 2022 at the Royal Children's Hospital, Melbourne or Perth Children's Hospital, Western Australia. Patient episodes of transplant were identified using the Australian bone marrow transplant registry and Australian Bone Marrow Database registry (ABMDR). Relevant patient demographic, transplant and pulmonary complication episode data were collected retrospectively from both the electronic medical record (EMR) and written medical records. Local institutional ethics committee approval was obtained at the Royal Children's Hospital for this study, HREC reference 91777.

Pulmonary complications were defined as signs and symptoms of pulmonary disease (including tachypnoea,

respiratory distress, fever, hypoxia or haemoptysis) and newly developed pulmonary imaging changes [chest x-ray (CXR) or computed topography (CT)] or changes in lung function following allogeneic HCT. Complications were classified as occurring early (first 100 days of post-transplant) or late (after 100 days of post-transplant). Patients were included if they required admission to either the day oncology unit or inpatient ward setting. Pulmonary complications were then further classified as either infectious, non-infectious complications or a combination of both. Infectious complications were classified as either a microbiologically defined infection (MDI) or clinically defined infection (CDI).[26] A MDI was defined as an infection that is clinically detectable and microbiologically proven. Causes of bacterial pneumonia were identified on direct BAL sampling and on peripheral blood cultures. A CDI was defined as a site of infection that is diagnosed but its microbiological pathogenesis cannot be proven or is inaccessible to examination. Non-infectious complications were defined using definitions from the American Thoracic Society, National Institute of Health (NIH) and expert consensus definitions and are detailed in Supplementary table 1.[6,8,27] Pulmonary complications were classified as severe based on the following outcome data: if a patient required oxygen therapy, intensive care admission, developed chronic lung disease or died because of the pulmonary complication. Outcomes of pulmonary complications were also measured longitudinally at 1, 3 and 6 months post onset of symptoms. Children were followed up for a minimum of 8 months or until transitioned to an adult centre or until the end of 2022 whichever occurred latest. General transplant outcomes including rates of acute GVHD (Seattle criteria[28]), chronic GVHD (NIH criteria[29]), veno-occlusive disease (VOD) (EBMT Paediatric Criteria[30]) and transplant-associated thrombotic microangiopathy (TA-TMA) (Modified Jodele criteria[31]) were also collected.

Infection prophylaxis

Patients in this study were admitted in high efficiency particulate absorbing (HEPA) filtered, positive pressure single rooms for the duration of the conditioning regimen up until a minimum of 30 days post Day 0. Data were collected on initial antifungal, antiviral and antibiotic prophylaxis [including for *Pneumocystis jirovecii* pneumonia (PJP)] prescribed at the time of admission for HCT. Investigation for infection included bacterial microscopy and culture, fungal culture and viral respiratory polymerase chain reaction (PCR). Respiratory multiplex PCR panel included the following: rhinovirus, coronavirus, mycoplasma pneumonia, bordetella pertussis, human metapneumovirus, adenovirus, human parainfluenzae 1–4, parechovirus, enterovirus, respiratory syncytial virus and influenza A and B. Fungal infections were classified as proven, probable and possible according to EORTC criteria.[32]

Data analysis

Statistical analyses were performed in GraphPad Prism version 10. Qualitative variables are described as numbers and percentage (%), and continuous variables are reported as medians and interquartile range (IQR). The pre-transplant and transplant characteristics were compared between patients who developed a pulmonary complication or not, via Fisher's exact *t*-test for categorical variables and the Mann–Whitney *U*-test for continuous variables. Univariate analysis to determine relative risk and 95% confidence interval was calculated using Koopman asymptotic score. This was based on characteristics pre and during the first transplant episode only and is visualised using the Forest plots including 95% confidence intervals. Pulmonary complication types and characteristics were collected on patients following the first transplant episode through and inclusive of the second transplant episode (if applicable) up until the end of 2022, or until the patient was transferred to another centre for example in the setting of transition to adult care or death. Survival rates were estimated using the Kaplan–Meyer method, differences were assessed using the log rank test, and hazard ratio was calculated using the Mantel–Haenszel method. For all analyses, a P-value < 0.05 was considered significant.

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AUTHOR CONTRIBUTIONS

Hannah Walker: Conceptualization; data curation; formal analysis; methodology; project administration; resources; visualization; writing – original draft; writing – review and editing. **Joanne Abbotsford:** Data curation; writing – review and editing. **Gabrielle M Haeusler:** Conceptualization; methodology; supervision; writing – review and editing. **Daniel Yeoh:** Conceptualization; methodology; supervision; writing – review and editing. **Shanti Ramachandran:** Methodology; supervision; writing – review and editing. **Michelle Ng:** Writing – review and editing. **Jonathan Holzmann:** Data curation; writing – review and editing. **Shivanthan Shanthikumar:** Methodology; supervision; writing – review and editing. **Heather Weerdenburg:** Data curation; writing – review and editing. **Diane Hanna:** Methodology; supervision; writing – review and editing. **Melanie R Neeland:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; supervision; visualization; writing – original draft; writing – review and editing. **Theresa Cole:** Conceptualization; methodology; supervision; writing – review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author.

These data are not publicly available because of privacy or ethical restrictions.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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REVIEW

Biomarkers to predict and diagnose pulmonary complications in children post haematopoietic stem cell transplant

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Abstract

Objectives. Haematopoietic cell transplant (HCT) is a cellular therapy for a group of high-risk children with cancer, immunodeficiency and metabolic disorders. Whilst curative for a child's underlying condition, HCT has significant risks associated, including lung injury. These complications are associated with increased post HCT mortality and require improved methods of risk stratification, diagnosis and treatment. Methods. Biomarkers measured in bronchoalveolar fluid and peripheral blood have been identified for both acute and chronic lung injury post HCT.This review evaluates the current research available investigating the use of these biomarkers to improve clinical care, with a focus on the paediatric cohort. Results. Elevated levels of cytokines such as IL-6, IL-8, G-CSF and TNF were identified as potential predictive biomarkers for the development of post HCT lung disease. The pulmonary microbiome was found to have strong potential as a biomarker pre and post HCT for the development of pulmonary complications. General limitations of the studies identified were study design, retrospective or single centre and not exclusively performed in the paediatric population. Conclusion. To translate biomarker discovery into clinical implementation further research is required, utilising larger cohorts of children in prospective trials to validate these biomarkers and determine how they can be translated into better outcomes for children post HCT.

Keywords: haematopoietic stem cell transplant, paediatric, pulmonary

INTRODUCTION

Pulmonary complications after haematopoietic stem cell transplant

Haematopoietic cell transplant (HCT) is a cellular therapy used in children with a range of haematological conditions, immunodeficiency syndromes and metabolic disorders. Whilst HCT offers a cure for the child's underlying condition, it has a range of infectious and inflammatory complications that can occur, including pulmonary complications.[1] Pulmonary complications occur in approximately 25–60% of children following HCT and contribute to 25–65% of non-relapse mortality.[2,3]

Pulmonary complications are frequently classified as infectious or non-infectious, with the latter divided into acute or chronic lung injury determined by onset post HCT. Acute lung injury, classically occurring in the first 100 days post HCT, includes idiopathic pneumonia syndrome (IPS), defined[4] as widespread lung injury demonstrated on imaging and by respiratory symptoms, in the absence of a lower respiratory tract infection. Diffuse alveolar haemorrhage (DAH), thought to be a subset of IPS with predominant vascular endothelial dysfunction, also occurs in the early stages post HCT and portends a worse prognosis. Chronic lung injury post HCT classically occurs later than 100 days post HCT and includes diagnoses of bronchiolitis obliterans syndrome (BOS),[1,2] restrictive lung disease (RLD) and cryptogenic organising pneumonia. Due to advances in supportive care and treatment with broad-spectrum antimicrobials, outcomes from infectious pulmonary complications have improved in recent years.[5] In contrast, with more limited methods for early detection and targeted therapy, the prognosis for patients with non-infectious pulmonary complications remain poor.

The categorisation of infective versus non-infective pulmonary complications is often too simple to encapsulate the lung disease that occurs in children post HCT; alternatively, pulmonary complications are often the result of a series of cumulative insults. These insults arise from pre HCT treatments, disruptions to the pulmonary microbiome, infectious pathogens, endothelial dysfunction, dysregulated inflammation and graft factors particularly mismatched donor source[5–11]. As a result, multi-modal therapy, including antimicrobials, immune modulating agents and ventilatory support in the intensive care unit (ICU) is often required. Current methods to detect and diagnose pulmonary complications include direct sampling of the cells and organisms within the lung bronchoalveolar fluid, commonly via bronchoalveolar lavage (BAL). BAL fluid is considered representative of the cellular interactions at the alveolar level.[12] BAL is generally well tolerated, even in these unwell patients and is lower risk procedure than in surgical lung biopsy.[12] In addition to the microbiological investigations, functional and imaging modalities are used to evaluate lung injury post HCT; however, these tools often diagnose lung disease once severe and potentially irreversible.

Following a diagnosis of post HCT lung disease, morbidity and mortality is significantly increased. Pulmonary complications are a leading cause of non-relapse mortality and even in those who survive initially, their 10-year mortality rates are higher than those who do not develop pulmonary complications.[13] These adverse patient outcomes are likely due to many contributing factors, including the incomplete understanding of the pathophysiology, as well as inadequate treatments of lung complications. Moreover, early and tailored therapy to target underlying mechanisms responsible for the lung injury are lacking. There is an urgent need for improved strategies to identify patients at risk of pulmonary complications before irreversible lung damage occurs, as well as rational and more effective therapeutic options for prevention and treatment. Biomarkers offer a potential strategy to assist in the prediction, prognostication and understanding of the biological mechanisms contributing to post HCT lung disease to improve patient outcomes.

METHODS: SEARCH STRATEGY

This review examines the current body of evidence related to biomarkers to predict the development of lung disease and/or its severity, diagnose and treat children with lung injury post HCT.

Medline, Embase and PubMed databases were searched using the search terms (child or paediatric) AND (haematopoietic stem cell transplant) AND (bronchoalveolar lavage OR

biopsy OR lung OR virus or bacterial or fungal infection). The inclusion criteria were studies that involved paediatric participants undergoing HCT and the exclusion criteria were animal studies and those that did not specifically measure a biomarker. Studies were also excluded if they measured only functional (lung function studies, e.g. Spirometry) or imaging biomarkers (e.g. Parametric response mapping on computed topography). The initial number of studies identified in the search were 2796. These were reviewed initially by title and abstract and reduced to 201. The reference lists of these articles were also reviewed for suitable articles. In total, 19 articles fulfilled inclusion and exclusion criteria. The results were summarised in a narrative review, given the heterogeneity of biomarkers did not permit a meta-analysis. The included articles were divided into categories of acute and chronic lung injury post HCT. Table 1 summarises these biomarkers and at which timepoint in relation to transplant they have been utilised in the studies included in this review. Figure 1 highlights the key biomarkers and associated diseases in acute and chronic pulmonary complications post HCT.

RESULTS

Acute lung injury

There have been 10 studies identifying 12 different biomarkers for the prediction and diagnosis of acute lung injury. The key findings of these studies are detailed in Table 2. Several researchers[12,14–16] focused on general acute lung injury as the primary outcome rather than a specific infectious or non-infectious diagnosis.

Neutropenia as a prognostic marker

There is evidence that neutropenia, both in the pre and post engraftment period, can be used as a biomarker for post HCT pulmonary complications. The aplastic phase post HCT is characterised by neutropenia. This commonly persists for the first 3 weeks post stem cell infusion and is associated with a high incidence of infective complications. A systemic absolute neutrophil count of less than 1×10^9 L^{-1}, within 48 h of developing lung imaging changes, has been associated with an increased risk of mortality. A single-centre study, including both infective and non-infective complications, demonstrated a higher risk of death in children who were neutropenic when they developed their pulmonary complication.[15] The duration and severity of neutropenia was not specified in this study as potential contributing factors. Although it is well known that prolonged and profound neutropenia significantly increases the risk of infective complications. A relatively deplete pulmonary neutrophil count, measured on BAL fluid, has also been shown to be a potential diagnostic tool in the post HCT setting.[16] A different single-centre study showed relative neutrophil depletion in addition to predominant lymphocytosis was seen in children post HCT who developed lung disease than in those who received chemotherapy alone.[16] In addition to conditioning agents, systemic inflammation, mediated by cytokines IL-6 and TNF, is associated with bone marrow suppression and the development of neutropenia post HCT.[15]

Cytokines for prediction of early respiratory failure and severe lung disease

Cytokines, the soluble mediators of inflammation, produced to mediate downstream cellular effects, have been shown to predict the development of post HCT lung disease.[12,14,17] Cytokines in these studies were measured by ELISA and sampled in both peripheral blood and BAL fluid. An example is IL-6, a pro-inflammatory cytokine released by macrophages, fibroblasts, Th17 and B cells.[12] Increased levels of IL-6 measured in both pre HCT BAL[12] and early post HCT peripheral blood[14] were associated with the development of post HCT lung disease and the development of acute respiratory failure post HCT suggesting IL-6 is a strong candidate biomarker for post HCT lung disease. In addition, ST2/IL-33receptor elevated in the first week post HCT has been associated with early development of respiratory failure.[14] This pathway has been implicated in a variety of inflammatory disorders as a mediator of type 2 inflammatory response and IL-33 receptors have also been found on lung epithelium.[18]

Cytokines as biomarkers for prediction of specific respiratory diseases post HCT have also been investigated.[19] Influenza is a common respiratory virus and is associated with increased morbidity and mortality in immunocompromised patients.[19] Severe influenza, defined by ICU admission or development of pneumonia, has been associated with a peripheral blood cytokine

INTRODUCTION

Pulmonary complications after haematopoietic stem cell transplant

Haematopoietic cell transplant (HCT) is a cellular therapy used in children with a range of haematological conditions, immunodeficiency syndromes and metabolic disorders. Whilst HCT offers a cure for the child's underlying condition, it has a range of infectious and inflammatory complications that can occur, including pulmonary complications.[1] Pulmonary complications occur in approximately 25–60% of children following HCT and contribute to 25–65% of non-relapse mortality.[2,3]

Pulmonary complications are frequently classified as infectious or non-infectious, with the latter divided into acute or chronic lung injury determined by onset post HCT. Acute lung injury, classically occurring in the first 100 days post HCT, includes idiopathic pneumonia syndrome (IPS), defined[4] as widespread lung injury demonstrated on imaging and by respiratory symptoms, in the absence of a lower respiratory tract infection. Diffuse alveolar haemorrhage (DAH), thought to be a subset of IPS with predominant vascular endothelial dysfunction, also occurs in the early stages post HCT and portends a worse prognosis. Chronic lung injury post HCT classically occurs later than 100 days post HCT and includes diagnoses of bronchiolitis obliterans syndrome (BOS),[1,2] restrictive lung disease (RLD) and cryptogenic organising pneumonia. Due to advances in supportive care and treatment with broad-spectrum antimicrobials, outcomes from infectious pulmonary complications have improved in recent years.[5] In contrast, with more limited methods for early detection and targeted therapy, the prognosis for patients with non-infectious pulmonary complications remain poor.

The categorisation of infective versus non-infective pulmonary complications is often too simple to encapsulate the lung disease that occurs in children post HCT; alternatively, pulmonary complications are often the result of a series of cumulative insults. These insults arise from pre HCT treatments, disruptions to the pulmonary microbiome, infectious pathogens, endothelial dysfunction, dysregulated inflammation and graft factors particularly mismatched donor source[5–11]. As a result, multi-modal therapy, including antimicrobials, immune modulating agents and ventilatory support in the intensive care unit (ICU) is often required. Current methods to detect and diagnose pulmonary complications include direct sampling of the cells and organisms within the lung bronchoalveolar fluid, commonly via bronchoalveolar lavage (BAL). BAL fluid is considered representative of the cellular interactions at the alveolar level.[12] BAL is generally well tolerated, even in these unwell patients and is lower risk procedure than in surgical lung biopsy.[12] In addition to the microbiological investigations, functional and imaging modalities are used to evaluate lung injury post HCT; however, these tools often diagnose lung disease once severe and potentially irreversible.

Following a diagnosis of post HCT lung disease, morbidity and mortality is significantly increased. Pulmonary complications are a leading cause of non-relapse mortality and even in those who survive initially, their 10-year mortality rates are higher than those who do not develop pulmonary complications.[13] These adverse patient outcomes are likely due to many contributing factors, including the incomplete understanding of the pathophysiology, as well as inadequate treatments of lung complications. Moreover, early and tailored therapy to target underlying mechanisms responsible for the lung injury are lacking. There is an urgent need for improved strategies to identify patients at risk of pulmonary complications before irreversible lung damage occurs, as well as rational and more effective therapeutic options for prevention and treatment. Biomarkers offer a potential strategy to assist in the prediction, prognostication and understanding of the biological mechanisms contributing to post HCT lung disease to improve patient outcomes.

METHODS: SEARCH STRATEGY

This review examines the current body of evidence related to biomarkers to predict the development of lung disease and/or its severity, diagnose and treat children with lung injury post HCT.

Medline, Embase and PubMed databases were searched using the search terms (child or paediatric) AND (haematopoietic stem cell transplant) AND (bronchoalveolar lavage OR

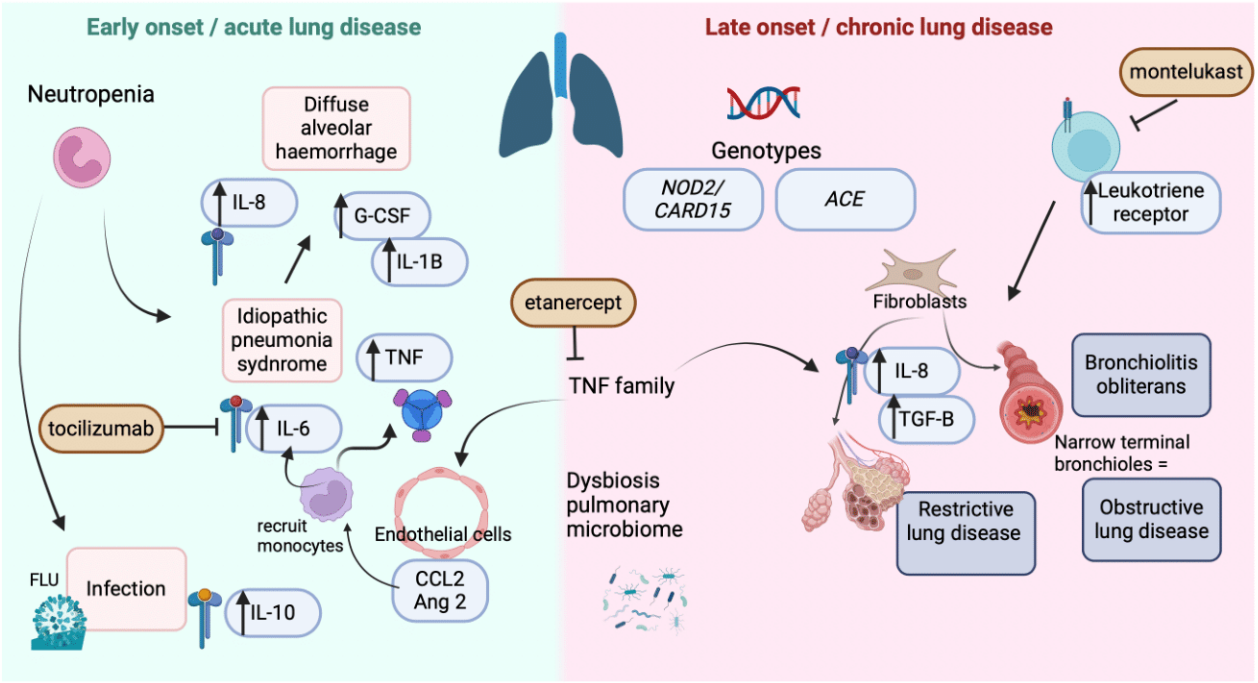

Figure 1. The key biomarkers and associated causes of acute and chronic pulmonary disease post-transplant. The crossover between the TNF family in causes of both acute and chronic lung disease is demonstrated. Therapeutic targets with clinical data supporting the potential use are also shown, for example, montelukast and etanercept. Biorender.com was used in the drafting of this figure. HCT, haematopoietic cell transplant; TNF, tumour necros is factor.

given the differing direction of association with influenza severity, it may be that IL-10 production is being driven by alternate cells.

Cytokines associated with idiopathic pneumonia syndrome

A severe manifestation of non-infectious or 'allo-immune' acute lung injury is IPS. IPS is one of the most widely investigated examples of a disease specific model for the use of clinical biomarkers post HCT and how these can be translated into targeted treatment. Several studies[20–22] have identified biomarkers of innate immunity, endothelial damage and systemic inflammation contributing to the development of IPS, with the strongest association being tumor necrosis factor alpha (TNF).[22,23] TNF is released following tissue damage secondary to conditioning agents such as total body irradiation (TBI) and in the setting of acute graft versus host disease (aGVHD).[24] Elevated levels of TNF and its soluble receptor TNFR1, measured in BAL fluid and plasma post HCT, in patients who develop IPS, have been demonstrated in a both a small discovery cohort[22] and larger paediatric cohort in comparison to controls.[23] C-C motif ligand 2 (CCL2) also referred to as MCP-1, monocyte chemoattractant protein, is released from endothelial cells in response to TNF and IL-1B.[21] MCP-1 functions to recruit monocytes and fibroblasts to sites of inflammation, for example, the lung in the case of IPS.[21] Elevated levels of MCP-1 in both BAL and plasma have also been implicated as a mechanistic biomarker in children with IPS than unaffected children.[21,22] Another mediator of endothelial activation and impending tissue damage is the protein Ang-2, which is expressed on endothelial cells and functions to sensitise cells to TNF.[23] Ang-2 has been shown to be raised in children with IPS in plasma at diagnosis and also to correlate with response to

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Table 2. Summary of research biomarkers investigating acute lung injury

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Hildebrandt *et al.* 2004[21]	BAL fluid at the time of developing pulmonary complication	Prospective, single centre *N* = 30 Adult and paediatric	CCL2/MCP-1 ELISA	IPS defined as diffuse lung injury in the first 100 days post HCT in the absence of infection	11 patients who developed IPS had significantly elevated CCL2/MCP-1 levels ($P < 0.05$) in BAL fluid than patients with chronic post HCT lung disease ($n = 14$) and normal controls ($n = 5$)
Yanik *et al.* 2008[22]	BAL fluid and peripheral blood	Prospective, multicentre Patients diagnosed with IPS *N* = 15 Adult and paediatric Control groups BAL cytokines $n = 16$ and plasma cytokines $n = 16$	TNF MCP-1 TNFR1 TNFRII IL-6 sCD17 LBP ELISA	Response to etanercept, defined as time to decreased oxygen requirement and survival at 28 days Response of inflammatory cytokines; systemic and pulmonary to treatment	Response to etanercept was seen in 13/15 patients. Survival at day 28 was 73% Elevated BAL and plasma levels in patients with IPS than controls; TNF, TNFR1, IL-6, sCD14, MCP-1 (CCL2) $P < 0.01$
Yanik *et al.* 2015[23]	Peripheral blood at onset of IPS and weekly for 4 weeks	Multicentre Phase II; prospective Trial *N* = 28 Paediatric Control groups $n = 9$ (combination of healthy patients and children post HCT without lung complications)	IL-8 IL-6 Ang-2 LBP sTNFR1 sTNFR2 LBP ELISA	Response to etanercept, defined as time to ceasing oxygen therapy and survival at day 28 and 1 year. Plasma inflammatory cytokines	Response in 71% (20/28) to etanercept Overall survival at 28 days 89% (CI 70–96) and 1 year 63% (CI 42–79%) TNFR1, used as a surrogate for TNF was increased in plasma of patients at IPS dx $P < 0.001$ than control group Significantly higher levels of; IL-6, IL-8, Ang-2, LPB and sTNFR2 at diagnosis of IPS $P < 0.01$ Responders to etanercept showed decline in plasma sTNFR1, IL-8 and Ang-2 than non-responders, not statistically significant
Whittle *et al.* 2001[12]	BAL fluid containing alveolar macrophage population pre HCT and post HCT	Prospective, single centre *N* = 34 adult & children Comparing patients with ($n = 11$) and without ($n = 21$) post HCT lung disease	TNF GM-CSF IL-6 ELISA Cytokines reported as a proportion of AM in cell culture	Post HCT lung disease defined as new radiographic opacification, or sustained tachypnoea with a fall in oxygen saturation > 5% from baseline on two readings, within 6 months from HCT	Median increase in cytokine levels in post HCT lung dx group than patients who did not develop pulmonary complications • TNF: 3 ng 10^{-6}AM (95% CI 0.1–7), $P = 1.01$ • GM-CSF: 4 ng 10^{-6} AM, $P = 0.006$, 95% CI = 0.5–7 IL-6: 0.3 ng 10^{-6} AM, CI 0.0002–1 $P = 0.049$
Kharbanda *et al.* 2006[17]	BAL fluid pre HCT	Prospective single site, *N* = 48 children, MPS Comparing patients with ($n = 25$) and without ($n = 23$) lung disease	IL-1β IL-8 G-CSF ELISA	Post HCT lung complications defined as either infectious or non-infectious by two study investigators based on clinical, radiological and bronchoscopic findings	Higher (mean) cytokine levels in post HCT lung dx group than the children who do not develop pulmonary complications post HCT • IL-β (104.2 picogramper g total protein versus 69.9 $P = 0.69$), • IL-8 (4327.2 versus 3155.7, $P = 0.052$) • GCSF (1022.3 versus 397.0 $P = 0.16$)

(Continued)

Table 2. Continued.

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Rowan *et al.* 2018[14]	Peripheral blood post HCT, Day + 7	Retrospective single centre, n = 122 (63 children) Comparing n = 22 patients who developed respiratory failure to n = 100 who did not post HCT	ST2 (IL-33 receptor) IL-6 ELISA	Respiratory failure within 30 days post HCT	Elevated ST2 (IL-33 receptor) and IL-6 at day 7 post HCT was associated with acute respiratory failure within 30 days, OR 8.9 (2.8, 28.6) (95% CI, $P < 0.001$) and 11.9 (2.6, 53.8) (95%CI, $P < 0.01$)
Choi *et al.* 2017[15]	Peripheral blood within 48 h of respiratory infiltrates on imaging post HCT	Retrospective observational, single site N = 35 children	Neutropenia	Mortality due to post HCT lung disease (up to 3 years post HCT) Pulmonary complication defined as the pulmonary infiltrates on chest imaging simultaneously with respiratory symptoms requiring admission, evaluation, or treatment	ANC $< 1 \times 10^9$ L^{-1} (OR 10.5, 95% CI, 1.07–104.65, $P = 0.044$) was associated with increased mortality post HCT
L'Huillier *et al.* 2019[19]	Peripheral blood post HCT at the time of Influenza diagnosis	Multicentre prospective observational study N = 277 (both paediatric and adult) Comparison of serum levels in n = 277 patients at two timepoints post HCT	Luminex 100 system Th1: IFN-y Th2: IL-13	ICU admission or development of pneumonia related to Influenza post HCT	Lower Th1:Th2 ratio at Day 0 associated with reduced ICU and at Day 28 associated with reduced ICU admission and pneumonia development. $P < 0.05$
Rochat *et al.* 2008[16]	BAL in symptomatic patients post HCT	Retrospective case control, single centre N = 19 Comparing n = 9 children post HCT to n = 10 children who received chemotherapy alone and developed pulmonary complications	Lymphocyte count Total and differential cell count; presence of cellular atypia Differences in the mean percentage cellular composition patterns between the two groups	Respiratory complications defined as rapidly progressing respiratory symptoms and radiological findings (new or persistent infiltrates on CXR or CT)	Post HCT pulmonary disease group than the chemotherapy alone group had • Predominant lymphocytosis (18% versus 6.25%, $P = 0.03$), • Higher levels of atypical epithelial cells (75% versus 30.8%, $P = 0.027$) • Neutrophil deplete (25.9% versus 58%, $P = 0.02$)
Seo *et al.* 2018[20]	Peripheral blood post HCT in patients with pneumonia/IPS/ Controls	Retrospective Single centre, case control N = 41 children and adults Comparing 41 patients who developed IPS to a cohort of n = 162 controls (no infection post HCT) and n = 37 who developed viral infection	ELISA IL-6 ST2 TNFR1	Diagnosis of Idiopathic pneumonia syndrome than viral pneumonia	Elevated ST2 and IL-6 in IPS post HCT versus controls OR 2.8 (2.0–4.0, 95% CI, $P < 0.001$) and 1.4 (1.0–1.9, 95% CI, $P < 0.025$) Elevated TNFR1 in IPS than viral pneumonia at onset of respiratory symptoms, OR 2.9 (1.5–6.0, 95% CI, $P < 0.001$)

AM, alveolar macrophages; BAL, bronchoalveolar lavage; HCT, haematopoietic cell transplant; IPS, idiopathic pneumonia syndrome; LBP, lipopolysaccharide binding protein; MPS, mucopolysaccharidoses.

targeted therapy, with etanercept.[23] Etanercept, a soluble TNF inhibitor, previously used in other inflammatory conditions such as rheumatoid arthritis, was trialled as a treatment for IPS.[22] A paediatric multicentre phase II trial showed clinical response in 71% of children treated with etanercept, when used in combination with corticosteroids, with overall survival of 63% at 1 year.[23] This is compared to historical cohorts prior to etanercept which reported survival of only 20–50% in patients who develop IPS post HCT.[25,26] Similar to general acute lung injury, IL-6 has also been shown to have specificity to IPS as a diagnostic biomarker[20,22,23] at the time of developing respiratory symptoms post HCT. Overall, IPS is a landmark example of how diagnostic biomarkers can not only be utilised for therapeutic targets but also highlights the need for ongoing strategies to improve sustained survival in non-responders.

Diffuse alveolar haemorrhage

A subset of IPS, DAH is a rare complication post HCT but is associated with high mortality.[17] DAH has been observed at higher rates in children with a diagnosis of metabolic storage disorders such as mucopolysaccharidoses (MPS), making them a cohort of particular need of predictive biomarkers.[17] Elevated levels of IL-1B, IL-8 and G-CSF in bronchoalveolar fluid of children pre HCT was seen in the group of children who developed post HCT lung complications, including DAH which occurred in 19% of these children.[17] IL-1B IL-8 and G-CSF are pro-inflammatory cytokines involved in T cell stimulation, chemotaxis and activation of monocytes and neutrophils.[17] This suggests an increased risk signature pre HCT in this group of children, who are relatively 'treatment naïve' in comparison to their counterparts who undergo HCT for a malignant indication.

Chronic lung injury

There have been nine studies exploring eight different biomarkers for the prediction and diagnosis of chronic lung injury. The key findings of these studies are detailed in Table 3.

Bronchiolitis obliterans

One of the most common types of non-infectious chronic lung disease post HCT is BOS, a pulmonary manifestation of chronic graft versus host disease (cGVHD).[27] BOS is diagnosed in adults using pulmonary function tests to demonstrate obstructive lung disease (OLD) and imaging studies showing air trapping, in the absence of infection.[28,29] In children, pulmonary function testing is more challenging and often not reproducible or possible in those aged less than 7 years.[30] This is problematic as BOS is often asymptomatic until significant airflow obstruction has occurred and conversely once symptomatic the differential diagnosis is broad, including infection and alternative lung pathology. This highlights the need for alternative and complementary biomarkers for diagnosis of BOS.

Leucotriene receptor levels have been shown in adult and paediatric populations to be elevated in both the peripheral blood and urine of patients who have been diagnosed with BOS, than healthy controls.[31] Leucotrienes are responsible for mediating multiple immune pathways; of relevance in BOS this is thought to be related to activation of fibroblasts, leading to collagen deposition and obstruction of terminal bronchioles.[31] This led to the use of montelukast therapy, which blocks this pathway, for treatment of BOS. A Phase II trial[31] of patients treated with montelukast therapy showed a decrease in leucotriene receptor levels in peripheral blood monocytes and neutrophils and functional improvement with slower decline of forced expiratory volume in 1 s (FEV1). Whilst this was a small cohort of patients, it is an example of the translation of a biomarker to a therapeutic target and correlation to functional studies (spirometry).

Restrictive lung disease

Restrictive lung disease can also be a manifestation of chronic GVHD, with TBI being a known risk factor.[32] In contrast to OLD, RLD post HCT is associated with reduced forced vital capacity (FVC) and total lung capacity (TLC). Whilst OLD and RLD differ in terms of functional assessment and pulmonary imaging, similar patterns of elevated plasma biomarkers (TNFR1, TNFR2, IL-8 and TGF-B) have been observed post HCT than patients without chronic lung disease.[33] This suggests that cGVHD culminating in end-stage fibrosis have shared biomarker profiles despite different anatomical involvement, that is, in terminal airways in OLD than the interstitium in RLD. As a result, etanercept, was trialled as a

Table 3. Summary of research biomarkers investigating chronic lung injury

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Versluys *et al.* 2018[40]	Paired BAL and NPA pre HCT	Retrospective Single centre *N* = 179 children Comparing patients with respiratory virus *n* = 110 to *n* = 69 who did not have a respiratory virus pre or post HCT	Viral respiratory panel compared NPA to BAL findings PCR assays	Post HCT lung complications, defined as Idiopathic pneumonia syndrome or bronchiolitis obliterans as defined by the American Thoracic Society; IPS = evidence of widespread lung injury by clinical symptoms and radiological abnormalities in the absence of LRTI and other factors to explain pulmonary dysfunction (cardiac dysfunction, fluid overload or renal failure) BOS = NIH 2014; FEV1/VC ratio of < 0.7, FEV1 of < 75% and evidence of air trapping (on PFTs or HRCT)	Virus detected pre HCT on BAL predictive of alloimmune lung disease: HR, 3.8 (95% CI, 1.4–10.7, *P* = 0.01) Virus detected on pre HCT NPA was not predictive of post HCT lung disease
Versluys *et al.* 2010[39]	NPA swabs post HCT	Prospective single centre *N* = 110 children	Respiratory virus PCR on NPA	Development of alloimmune lung disease acute (IPS) or chronic (BO/BOOP) allo lung dx	Respiratory virus infection early post HCT was predictive of allo-immune lung disease (*P* < 0.0001) BOS; HR = 107; 95% CI (0.9–13 347, *P* = 0.05) IPS; HR = 11.4; 95% CI (2.61–49.8 *P* = 0.01) Grade II-IV aGVHD post HCST was protective in relation to development of allo-immune lung disease
Williams *et al.* 2022[31]	Peripheral blood, urine and BAL	Phase II, prospective multi centre *N* = 23 s mod-severe lung disease Adult and paediatric Compared to historical cohort of healthy patients	Leucotriene receptor levels on blood eosinophils and monocytes and BAL at enrolment Flow cytometry on whole blood Mass spectrometry on BAL	To assess whether montelukast altered lung decline for patients with established BOS FEV1 stability or improvement (≤ 15% decline) at 6 months of montelukast therapy Leucotriene receptor activity	Elevated leucotriene receptor levels on blood eosinophils (*P* = 0.018) and monocytes (*P* = 0.027) in BOS patients versus healthy controls. These decreased with treatment to montelukast therapy Leucotriene receptors were not detected in BAL samples 91% of (21/23) patients met criteria for FEV1 stability or improvement after 6 months of therapy
Miyamoto *et al.* 2014[35]	Peripheral blood pre HCT	Retrospective single centre *N* = 149 (adult and children) Comparing *n* = 18 who developed non-infectious pulmonary complications and *n* = 131 who did not develop NIPC post HCT	*ACE* genotype ACE enzyme PCR amplification & ELISA	Development of non-infectious pulmonary complications post HCT NIPC was defined as evidence of widespread alveolar injury in multilobar infiltrate, and clinical symptoms of pneumonia and evidence of abnormal respiratory physiology, for example, impairment on pulmonary function test	*ACE* D/D (deletion/deletion) genotype, increased risk of developing non-infectious pulmonary complications post HCT, HR 8.8, *P* < 0.001

(Continued)

Table 3. Continued.

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
Yanik *et al.* 2012[33]	Peripheral blood	Prospective, single centre Patients with obstructive or restrictive lung dx post HCT *N* = 34 Comparing to controls post BMT Adult and paediatric	Plasma cytokine levels ELISA	Primary outcome: Response to etanercept related to FEV1 or FVC improvement (10% increase) at 4 weeks To determine plasma biomarkers for diagnosis and tracking therapy response to etanercept	33% of patients met primary outcome, improvement in lung function at 4 weeks but this was not statistically significant $P = 0.73$ Elevated TNFR1, TNFR2, IL-8, *TGF-β* in plasma of patients with OLD or RLD
Zinter *et al.* 2024[37]	BAL fluid at the time of pulmonary complication post HCT	Prospective, multicentre *N* = 229 children 278 BAL samples collected at the time of pulmonary complication; underwent analysis of pulmonary microbiome, immune cell gene expression and mortality Validation cohort 57 children from the Netherlands	Metatranscriptomics of pulmonary microbiome and human gene expression of immune cells	Key outcomes; Patient cluster and overall survival Patient subtypes classified as; Cluster 1: High diversity of commensal microbiota, predominance of antigen presenting cells (alveolar macrophage) Cluster 2: Increase in bacterial organisms, increased neutrophil infiltration and activation Cluster 3: Depletion of commensal microbes, increased viral and fungal pathogens, higher proportion of lymphocytes with activation of fibroproliferative pathways Cluster 4: Depletion of commensal microbes, increased S. aureus and viral pathogens, higher proportion of lymphocytes	4 key clinicopathological clusters of patient subtype based on BAL sample analysis were identified and prognostic of patient survival Diverse pulmonary microbiome was the most commonly seen (Cluster 1) associated with the lowest mortality ($P = 0.005$) Deplete pulmonary microbiome and enrichment of viral pathogens (Cluster 3 &4); were associated with higher mortality ($P = 0.005$)
Zinter *et al.* 2022[11]	BAL fluid and paired pulmonary function tests pre HSCT in asymptomatic children	Prospective single centre *N* = 104 children, the Netherlands	Metatranscriptomics of pulmonary microbiome and human gene expression of immune cells PFT grouping related to lung capacity, obstruction, diffusion and air trapping	To determine if there is an association between pulmonary microbiome and PFTs patterns, for example, obstruction or restriction pre HSCT and all-cause mortality post HCT	Reduced FVC% pred was associated with post HCT mortality, HR 1.21(1.03–1.43, $P = 0.024$) 95% CI Multivariate analysis; Reduced lung capacity, pre HSCT is associated with a pulmonary microbiome that is deplete, suggest a pro-fibrotic immune signature and predicts higher post HCT mortality
Zinter *et al.* 2021[36]	BAL fluid collected pre HSCT	Retrospective, single centre *N* = 181 children, the Netherlands 181 BAL samples for analysis collected prior to HCT as part of clinical care for another reason, for example, Central line insertion in children who did not have clinical suspicion for a lung infection	Metatranscriptomics of pulmonary microbiome and human gene expression of immune cells	Key outcomes: Development of post HCT lung injury Non-relapse mortality and fatal lung injury BAL metatranscriptome clusters: Cluster 1: Oropharyngeal heavy (microbiota rich) Cluster 2: Microbially deplete Cluster 3: Oropharyngeal light Cluster 4: Virus enriched	Lung injury Microbially deplete and virus enriched microbiome associated with increased development of post HCT lung injury; HR 95% CI 5.6 (2.5–12.8, $P < 0.001$) and 3.5 (0.9–13, $P = 0.06$) Fatal Lung Injury

(Continued)

Table 3. Continued.

Author	Biological source	Study details	Biomarkers and platform	Primary outcome and key definitions	Key result
		Validation cohort 18 children; underwent BAL for a clinical indication			Microbially deplete and Virus enriched microbiome associated with increased fatal lung injury; HR 95% CI 6.8 (1.9–24.3, $P = 0.003$) and 6.3 (1.1–38.2 $P = 0.044$)
Hildebrandt *et al.* 2008[28]	Peripheral blood	Multicentre, prospective $N = 427$ Adult and paediatric	Blood of both donor and recipient pairs, DNA, SNP array	Development of BO and differences with specific donor/recipient *NOD2/CARD15* SNP mutations than wildtype BO defined using NIH criteria	Recipient or donors with *NOD2/CARD15* SNP variants (SNP8, SNP12, SNP13) are associated with increased development of BO in 18.7% ($P < 0.001$) than 1.3% incidence of BO without these mutations (wildtype) Recipient mutations had a stronger association than donor mutations in multivariate analysis

aGVHD, acute graft versus host disease; AM, alveolar macrophages; BAL, bronchoalveolar lavage; BO, bronchiolitis obliterans; BOS, bronchiolitis obliterans syndrome; FEV, forced expiratory volume; FVC, forced vital capacity; HCT, haematopoietic cell transplant;HRCT, High resolution Computed topopgraphy; HSCT, Haematopoietic stem cell transplant; IPS, idiopathic pneumonia syndrome; LBP, lipopolysaccharide binding protein; MPS, mucopolysaccharidoses; NIH, National institute of health; NPA, Nasopharyngeal aspirate; OLD, obstructive lung disease; PFT: Pulmonary function test.

therapeutic target and while a small cohort of patients demonstrated improvement in both obstructive and restrictive patterns of spirometry, there was no statistically significant difference in overall survival.[34]

Genotypes to predict risk of chronic lung disease

In addition to soluble biomarkers, two studies[28,35] have identified genetic variations that may influence the development of lung disease post HCT, in angiotensin converting enzyme (ACE) and nucleotide binding oligomerisation domain containing 2/caspase recruitment domain family, member 15 (*NOD2/CARD15*). The ACE enzyme is active in lung endothelium and functions to produce Angiotensin II, a protein that acts on pulmonary fibroblasts, shown to contribute clinically to pulmonary hypertension and fibrosis.[35] Specific variations in the *ACE* genotype have been associated with an increase in non-infectious lung disease post HCT (obstructive and restrictive).[35] By comparison, the *NOD2/CARD15* gene is expressed on lung epithelial cells, as well as circulating monocytes and macrophages.[28] Mutations in the *NOD2/CARD15* gene were associated with a statistically significant higher incidence of BOS development than patients without this mutation (wildtype).[28] Both studies[28,35] identified patients with genetic biomarkers that, via different pathways, lead to alloreactive immune dysregulation toward a common pathway of pulmonary fibrosis.

Pulmonary microbiome pre and post HCT

There have been three studies from the same group that explored the relationship with pulmonary microbiome and chronic lung disease in paediatric HCT. The ability to describe patterns of pulmonary microbiome signature or 'cluster', on BAL samples, has been shown to have potential as a biomarker both pre and post HCT for the development of post HCT lung injury.[11,36] The finding that the lung, similarly to the gastrointestinal tract, is not sterile and host to wide array of micro-organisms, has been demonstrated in several paediatric studies.[11,36,37] Initially identified in a retrospective cohort,[36] it was shown that children with pulmonary microbiome clusters pre HCT that were either deplete of commensal microbiota (cluster 2) or

viral enriched (cluster 4) had higher rates of post HCT lung injury and subsequent death. A subset of these children also had matched pre HCT pulmonary function tests performed.[11] Poor pulmonary function pre transplant has been shown to be independently associated with worse post HCT outcomes in terms of non-relapse mortality.[38] The correlation of pulmonary microbiome data with pulmonary function tests identified an abnormal microbiome, defined as deplete of common commensal organisms, was associated with reduced lung capacity (FVC% pred).[11] The immune profile of the BAL fluid in these patients with microbiome dysbiosis was also associated with a pro-fibrotic immune response, which may have contributed to the lower lung capacity seen in these children.[11] A prospective trial from the same group Zinter *et al*.[37] applied similar analysis techniques in children post HCT, with BAL samples collected to investigate a pulmonary complication. Again, this identified that a diverse pulmonary microbiome was protective and associated with lower mortality post HCT than a viral enriched, deplete microbiome.[37] A cohort of children in this study also required a second BAL for new or progressive pulmonary disease and this revealed a temporal shift of the microbiome to cluster as high risk.[37] This suggests ongoing pulmonary insults post HCT continue to influence the microbiome. Collectively these three studies[11,36,37] of the pulmonary microbiome demonstrate that microbiome dysbiosis may be associated with an increased risk of developing chronic post-transplant lung disease and increased mortality.

Influence of viral infection on chronic lung disease

Similar to the finding that a pulmonary microbiome enriched with viral organisms is associated with worse outcomes, the presence of viral infection, detected using quantitative PCR methods, has a role as a predictive biomarker for post HCT lung disease.[27,39,40] Respiratory virus pathogens detected on BAL fluid pre HCT[40] and nasopharyngeal swab early post HCT[39], have been shown to increase a child's risk of post HCT chronic lung disease.[40] This included viruses that did not cause significant or severe lung disease with initial infection, for example, Rhinovirus, highlighting the potential pathogenic role of these infections in promoting donor-mediated allo- reactive immune dysregulation. To understand the link between the presence of a virus and clinical outcome post HCT, more research is required into the individual cytokine response or gene expression profiles of the patients who have worse clinical outcome.

DISCUSSION

This review identified 19 studies that investigated the use of predictive, diagnostic and therapeutic biomarkers in children who develop post HCT lung complications. Within these studies, over 20 individual biological biomarkers in blood and BAL fluid were investigated. Cytokines, soluble mediators of inflammation and infection, were commonly identified as biomarkers for both acute and chronic lung complications. Broadly, interactions between cytokines and their receptors, contribute to leucocyte migration and are key to both adaptive and innate immune responses.[21] It is not surprising that many of the studies identified in this review investigated cytokines as key candidate biological biomarkers. In the case of HCT, the cellular immune responses become more complex due to the nature of transplant chimera. Specifically, the interactions between donor and recipient immune cell populations and the actions of cytokines produced by both the donor and recipient. In relation to pulmonary complications, it is plausible that the lung resident immune cells, for example alveolar macrophages, are influenced by a milieu of systemic cytokines from the donor as well as by recipient innate cells within lung tissue. One of the potential limitations of several studies reviewed is the difficulty in delineating the cellular 'source' of cytokine production, specifically, donor versus recipient. Over time, post HCT, the expanding immune cell populations from donor origin predominate, particularly when chimerism is $>$ 95%. This may be important to understand in the context of time post HCT and degree of immune reconstitution of the donor. There are many factors, including those between host and recipient that contribute to dysregulated inflammation in response to cumulative tissue injury from HCT.

It is well established that the development of lung complications posts HCT contribute significantly to paediatric intensive care unit admission and non-relapse mortality post HCT.[41] Several authors looked at pre HCT cytokines as

predictive biomarkers of mortality post HCT. The pre HCT cytokine levels in this setting would be produced by the patient (recipient) who has not yet received a HCT and suggests some predisposition that is potentially independent of the donor. Common cytokine patterns identified pre HCT that were predictive of post HCT disease were elevated IL-6 and GM-CSF levels.[12,14,20,22,23] In contrast, in the post HCT setting, the cytokine milieu is likely to be produced and influenced to a greater proportion by the engrafted donor immune cell population. In the lung, however, like other extramedullary organs, there is potentially more heterogeneity in the immune populations from the donor and recipient. Perhaps, it is this complex cellular interplay within the lung, which makes this organ a key target for the spectrum of post HCT complications observed in our patients.

The relationship between immune mechanisms in the lung post HCT has been extrapolated from patients who have required lung transplantation for severe BOS.[42,43] In patients who required a lung transplant for severe BOS post HCT, proinflammatory M1 alveolar macrophages of stem cell donor origin were the dominant immune cell type found in the explanted lungs of patients who underwent lung transplantation for post HCT BOS[42]. This is in comparison to a clinically similar phenotype of BOS post lung transplant in which the solid organ (lung) *recipient* alveolar macrophages were the dominant cell type responsible.[42] This highlights that both types of BOS share a similar clinical phenotype and pathophysiology, in both examples, the alveolar macrophage is of a different patient origin to the lung. It is likely that in future more can be learned from this patient group to design future biomarker studies in the paediatric post HCT cohort.

It is important to highlight that many of the studies identified in this review measured cytokines in cohorts of both adults and children undergoing HCT. This strategy, while allowing larger populations to be studied and improve statistical power, assumes that these populations are comparable. It is critical to note that key differences between children and adults in relation to immune physiology, transplant regimens and indications for transplant. These differences limit the applicability of biomarkers measured in a 'mixed' cohort unless validated in an exclusively paediatric cohort. Key differences in the HCT setting in paediatrics include the primary indication for HCT, intensity of conditioning regimens, cell graft source and types of pre-existing lung disease.[44] Children, compared to adults, have a higher percentage of non-malignant indications for HCT, such as inborn errors of immunity, metabolic disorders and haemoglobinopathies.[45,46] There is also an increased use of myeloablative-level conditioning and preference for grafts from bone marrow or cord blood in children. In contrast, adults are more likely to receive reduced intensity conditioning regimens and peripheral blood grafts. These differences may explain disparate treatment response between adult and paediatric cohorts in clinical trials for some lung complications, for example etanercept in IPS. It is also important to also acknowledge that children are not small adults with respect to their immune systems. In healthy individuals, immune cell proportions change with increasing age, including increased memory/naïve T cell ratio and decreased T cell repertoire related to thymic involution age.[47] These differences might explain contrasting clinical severity of infections with a notable example being less severe COVID-19 disease in children than adults.[48] This is a noteworthy principle when considering the applicability of immune cell proportions and cytokine profiles in adults as biomarkers in children. Robust knowledge translation also requires the establishment of normal reference ranges in adults and children pre HCT and post HCT.

This review also highlighted significant overlap in biomarkers, such as IL-6 and TNF family, across acute and chronic lung complications. This raises the question of whether these complications are not distinctly separate entities but a spectrum with shared biomarker patterns in response to similar pathophysiology. For example, tissue damage locally (both endothelial and interstitial), leading to cytokine release and T cell influx with dysregulated immune response. An example of this is the connection between acute GVHD, chronic GVHD and BOS. While it is established that aGVHD is a risk factor for cGVHD, the latter only occurs in a subset of children with prior aGVHD.[49] By comparison, BOS can occur in children without other manifestations of GVHD and this is poorly understood.[49] It is plausible that specific host and donor factors contribute to the unique end-organ vulnerability of the lung to the immune dysregulation of cGVHD. Furthermore, there is significant variability in the clinical response of patients with BOS to

treatment, with some patients failing systemic immunosuppression and progressing to lung transplantation than patients who have resolution of BOS who require minimal systemic therapy. Genetic biomarkers, such as variants in *NOD2/CARD15*, may help predict risk and are plausible contributory factors in severe and treatment refractory BOS.

At the time of this review, there were no published clinical data trialling the use of IL-6 inhibition, for example, tocilizumab in the treatment of post HCT lung complications. There is, however, a precedent for the use of tocilizumab for the treatment of both severe COVID-19 and cytokine release syndrome post CAR-T therapy, making it a rational targeted treatment with plausible benefit to study prospectively (ideally in randomised fashion) to prevent and/or treat post HCT lung disease.

The influence of the pulmonary microbiome in the development of post HCT lung disease holds significant promise in better understanding the variability of clinical outcomes seen.[11] This work by Zinter *et al*.[11,36,37] highlights the importance of identifying not only pathogenic but protective microbiome environments that correlate with the maintenance of lung health. These may then guide future therapeutic strategies, akin to those directed at the intestinal microbiome post HCT. The microbiome of the gastro-intestinal tract pre HCT has been shown to strongly influence rates of post HCT GVHD and non-relapse mortality.[50] Zinter *et al*.[11,36,37] have demonstrated in multiple cohorts that the pulmonary microbiome both pre and post HCT can similarly predict the development of pulmonary complications and associated non-relapse mortality. Further studies are required to identify potential strategies to manipulate the pulmonary microbiome pre HSCT in those with high-risk profiles. By comparison, there is less known about how to best recreate a 'healthy' or diverse pulmonary microbiome. It is likely, however, that much can be learnt from gastrointestinal microbiome research, including targeted immunotherapy (e.g. tocilizumab) and faecal microbiota transplant.[51,52] The finding that the pulmonary microbiome BAL clusters also evolved into high-risk groups in patients who had persistent pulmonary disease suggests a progressive natural history.[37] The relationship between the oropharyngeal, lung and gut microbiome has been extensively investigated outside the HCT setting and dysbiosis has been shown to be associated with increased inflammation and opportunistic infection.[53] How the 'gut-lung' axis is impacted by HCT and can be used as a biomarker for prediction and treatment of lung complications is an area of ongoing research.

Now is an ideal time to profile the inflammatory imprint of lung disease due to increasing experience with targeted immunotherapy to treat lung diseases than ever before. Key examples are the use of tocilizumab to treat severe COVID-19 lower respiratory tract infections, due to the finding of elevated IL-6 in BAL fluid[54,55] and biologics targeting IL-4 including dupilumab for severe Asthma.[56]

CONCLUSION

Pulmonary disease continues to contribute significantly to morbidity and mortality for children post HCT.[1,41] This review highlights the wide range of potential biomarkers to aid in the prediction, diagnosis and prognosis of children who develop these complications post HCT. The development of a biomarker that can translate into improved clinical outcomes for patients requires multiple steps from discovery to implementation, including validation and verification. The implementation of the most promising biomarkers in a time efficient and cost-effective manner requires incorporation into large multicentre prospective clinical trials. Many of the non-infectious post HCT pulmonary complications that occur are not well understood, have limited targeted therapeutic options and consequently poor outcomes. Biomarkers incorporated into clinical practice are key to helping us identify new therapeutic targets and hence improve survivorship for children undergoing allogeneic HCT. The use of a 'panel' of multiple biomarkers, using a range of methods, for example, BAL, peripheral blood, imaging and functional analysis, will best fulfil the requirements for this complex and heterogeneous group of patients rather than a single biomarker. A multidisciplinary group of experts highlighted this, calling for the identification of pathobiology-based biomarkers of BOS which can detect BOS in its earliest stages, prior to changes in lung function or lung imaging.[57] Overall, this review has shown that many potential candidate biomarkers have been identified but ongoing research is required to determine those that will meaningfully translate into clinical care to improve outcomes for our patients.

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AUTHOR CONTRIBUTIONS

Hannah Walker: Conceptualization; data curation; writing – original draft; writing – review and editing. **Gabrielle M Haeusler:** Conceptualization; supervision; writing – review and editing. **Theresa Cole:** Conceptualization; supervision; writing – review and editing. **Melanie Neeland:** Conceptualization; supervision; writing – review and editing. **Diane Hanna:** Conceptualization; supervision; writing – review and editing. **Shivanthan Shanthikumar:** Conceptualization; supervision; writing – review and editing.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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