Peripheral-blood cytopenia, an early indicator of inborn errors of immunity

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INTRODUCTION

Inborn errors of immunity are inherited monogenic defects resulting in dysregulation of the innate and/or adaptive immune response. The current prevalence is estimated at 4 to 10 per 100 000 live births per year with few studies describing the epidemiological features in Africa, less so in South Africa. Phenotypic presentation is heterogeneous and early recognition can be difficult. Both the International Union of Immunological Societies (IUIS) 2019 classification...
of IEI and the European Society for Immunodeficiency (ESID) have provided a clinical framework for classifying and understanding disease presentation.6,9

Although severe, unusual or recurrent infections are the most common presentation, up to 25% of IEI cases may be missed if clinical focus is on infection alone.10 Non-infectious manifestations are becoming increasingly more apparent.10–14 Severe, persistent and/or recurrent peripheral-blood cytopenia, the reduction of one or more mature blood cell lines, that is otherwise unexplained or unusual, may be a presenting laboratory feature or an observed secondary phenomenon.2,10,15,16 The recent IUIS 2019 addition of bone-marrow failure highlights the relevance of haematological manifestations in IEI.4

The pathogenic mechanisms of cytopenia in IEI are multifactorial. They are commonly attributed to cell-mediated and humoral immune dysfunction or dysregulation, driving autoimmunity and autoinflammation. However, non-immune causes should also be considered.2,15–17 B- and T-cell responses have a complex interdependent relationship, primary defects resulting in altered maturation and regulation with subsequent decreased number and function.2,10 Secondary factors such as splenic sequestration and bone-marrow infiltration by malignancy or infection are non-immune causes that should not be overlooked (Figure 1).17,21–23

FIGURE 1 Mechanisms contributing to cytopenia in inborn errors of immunity (IEI). (A) The pathogenic mechanisms of cytopenia are multifactorial and include: humoral and cell-mediated immune dysfunction and/or dysregulation, haemophagocytosis, splenic sequestration (secondary to abnormal lymphoproliferation), bone-marrow dysfunction including ineffective maturation, myelodysplasia, primary bone-marrow failure and myelosuppression due to secondary infections and bone-marrow infiltration.2,16 (B) Cytoskeletal dysfunction with megakaryocyte dysfunction, increased apoptosis and splenic platelet destruction. Contributory causes of non-immune thrombocytopenia in IEI include: increased clearance and apoptosis; myelosuppression by infections, myelofibrosis/bone-marrow malignancy, cytotoxic drugs or transplant are also contributory.1,16,18 (C) Defective myeloid cell differentiation or release of granulocytes from the bone marrow, enhanced apoptosis and increased destruction of peripheral-blood granulocytes are the main physiological mechanisms underlying chronic severe or intermittent neutropenia in primary immunodeficiency patients.1,16,20 (D) Lymphopenia driven by B- and T-cell-intrinsic defect with abnormal maturation and defective function, negative selection of autoreactive T lymphocytes in the thymus as well as defects in the number or function of regulatory T-cells, impaired apoptosis of autoreactive lymphocytes, loss or breakage of tolerance, increased lymphoid production and cytokine secretion which likely also drives the immune dysregulation and associated autoimmune cytopenia.2,25 [Colour figure can be viewed at wileyonlinelibrary.com]

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Inborn errors of immunity are increasingly more common than previously thought and require early identification for life-changing and/or lifesaving intervention.6,17,24 Understanding the non-infectious manifestations of IEI is important, to improve awareness of the wide diagnostic spectrum and facilitate precision drug targets.10 Definitive diagnostic investigations serve little purpose if the disease is not entertained at the outset. Identifying abnormalities of laboratory parameters that already form part of routine diagnostics, are readily available and that may facilitate earlier case identification, are paramount. The widely available full blood count (FBC) and leucocyte differential (DIFF), forming part of first-line investigation for suspected IEI, may facilitate earlier identification. This retrospective review of the South African Primary Immunodeficiency Registry (SAPIDR) aimed to assess the haematological indices at presentation; their association with IUIS 2019 classification for IEI; and mortality.

METHODOLOGY

Study design

This retrospective cross-sectional descriptive study spanning 2013 to 2020, was conducted in a tertiary state care facility in South Africa, utilising SAPIDR data. The IUIS 2019 IEI classification was applied and haematological parameters, at or within one month of diagnosing IEI, were assessed. SAPIDR patients undergo rigorous investigation to exclude secondary causes of immunodeficiency and have pre-existing signed informed consent. Data collection was granted by custodians. Ethical approval and waiver of consent was given by the Health Research Ethics Committee (S20/03/082).

Study procedures and measurements

Both state and private-sector laboratories must be accredited with the South African National Accreditation System (SANAS) and adhere to International Standards Organisation (ISO) ISO15189 requirements for medical laboratory quality. Molecular diagnosis was confirmed using Sanger Sequencing or next-generation sequencing (NGS) either through a focused IEI panel, whole-exome or whole-genome sequencing and results interpreted through multi-disciplinary discussion. Data were supplemented from the National Health Laboratory Service (N HLS) Laboratory Information System and hospital electronic clinical management database (Electronic Content Management). Information was captured on a Research Electronic Data Capture (REDCap®) form.25 Those without haematologi cal indices available were excluded. The evaluated parameters included patient demographics and laboratory indices, namely FBC, DIFF and other relevant haematological or immunological results.

Age-appropriate NHLS reference ranges were used for interpretation.26 Severe cytopenia was defined as; anaemia (haemoglobin) less than −2SD below age-appropriate reference range, thrombocytopenia less than 100 × 109/l, neutropenia less than 1.5 × 109/l and lymphopenia less than 3 × 109/l, persistent cytopenia lasting more than one month despite optimal management, unusual cytopenia with an otherwise unexplainable cause and recurrent or refractory
cytopenia as persistent despite optimal management.\textsuperscript{16,18,27,28} Pancytopenia was defined as absolute white cell count less than 4 \times 10^{9}/l, haemoglobin less than 100 g/l and thrombocytopenia less than 150 \times 10^{9}/l.\textsuperscript{29–31}

Statistical analysis

STATA 17 (College Station, Texas 77845, USA) was used for analysis. Descriptive statistics were performed on demographic and laboratory parameters, in relation to IUIS 2019 classification. Empirical diagnostic cut-off points that maximised sensitivity and specificity were determined for the neutrophil-to-lymphocyte ratio (NLR) using receiver operating characteristic (ROC) curves. Association between FBC parameters and IUIS classification was tested using the chi-squared test, or Fishers exact test. Odds ratios (OR) were reported as measures of association with the corresponding 95% confidence intervals (CI). The Kaplan–Meier method was used to analyse time until death from diagnosis, and comparisons were made with the log-rank test. Statistical significance was considered as \( p < 0.05 \).

RESULTS

Cohort characteristics

The SAPIDR includes both children and adults. Two thirds (65%, \( n = 257 \)) of patients on the SAPIDR (\( n = 396 \)) had available haematological indices, 34% (\( n = 136 \)) without haematological indices were excluded from the analysis. A molecular diagnosis with a pathological variant was identified in 30% (\( n = 77 \)) (Figure 2A). Those younger than 18 years made up majority (84%; \( n = 217 \)) of the cohort, of which 49% (\( n = 123 \)) were younger than five years while the remainder (16%; \( n = 40 \)) were older than 18 years. The median age at diagnosis was 76 months (range from birth to 420 months) with a median delay in diagnosis of 75 months (IQR = 30–174 months). Nineteen percent (\( n = 48 \)) presented within the first 12 months of life and 47% (\( n = 121 \)) below 60 months. Majority (54%, \( n = 138 \)) had a predominantly antibody deficiency (IUIS III). (Figure 2A) Those with cellular and humoral immune deficiency (IUIS I) and combined immunodeficiency (IUIS IIA) presented younger whilst those with IUIS III and complement deficiency (IUIS VIII) presented later in life (Table S1a). Presentation with cytopenia and age at diagnosis had a bimodal distribution, younger age (below 12 months) and older age most significant (Table S1a,b). The majority were of male sex (60%, \( n = 154 \)). Male sex was significantly associated with a molecular diagnosis (\( p = 0.004 \)) (Table S2).

Most (86% \( n = 223 \)) presented with severe, persistent, unusual and/or recurrent infection, 62% (\( n = 161 \)) were cytopenic at presentation and 27% (\( n = 71 \)) had a known family history of IEI, none were from a consanguineous relationship (Figure 2B). Haematological malignancy was observed in three cases, all with B-cell lymphoma; one with combined immunodeficiency (CID), one a hyper-IgM like syndrome and the other an undefined hypogammaglobulinaemia. Autoimmunity was seen in seven patients (3%), two cases had undefined hypogammaglobulinaemia, Aicardi Goutieres syndrome (\( n = 2 \)), IgA deficiency (\( n = 1 \)) and one a C6 deficiency (\( n = 1 \)). Only one patient with a complement deficiency (IUIS VIII) had documented haemolysis (Figure 2B).

FBC parameters

In those with cytopenia at presentation (\( n = 161 \)), 17% (\( n = 27/161 \)) were pancytopenic, 40% (\( n = 64/161 \)) anaemic, 8% (\( n = 13/161 \)) thrombocytopenic, 19% (\( n = 30/161 \)) neutropenic and 62% (\( n = 100/161 \)) lymphopenic (Figure 2B). Lowest median haemoglobin was seen in IUIS V and IUIS I, median platelet count was lowest in IUIS II, median lymphocyte count lowest in IUIS I and median neutrophil count lowest in IUIS III (Figure 3, Table S3). IUIS I diagnosis was significantly associated with cytopenia at presentation (OR 12.43, CI 3.82–39.05) namely; severe cytopenia (OR 3.96, CI 1.08–14.88), persistent cytopenia (OR 12.27, CI 3.75–39.95), and unusual cytopenia (OR 8.89, CI 3.04–25.86), namely; anaemia (OR 4.15, CI 1.58–10.90), leucopenia (OR 15.69, CI 1.86–132.29) and lymphopenia (OR 12.16, CI 2.75–53.73), where 52% (11/21) were anaemic, 38% (8/21) leucopenic and 90% lymphopenic (19/21). Cytopenia was associated with IUIS II (OR 5.99, CI 2.80–12.76) seen in 94% (31/33) namely; severe, persistent and/or unusual cytopenia (OR 3.58, CI 1.08–11.97), 3 (1.44–6.24) and 2.71 (1.32–5.60) respectively, 21% (7/33) were thrombocytopenic at presentation with significant association (OR 14.39, CI 2.89–71.57). Neutropenia was seen in 14% with IUIS III showing significant association (OR 3.65, CI 1.44–9.25) (Figure 4, Table S4). Pancytopenia at presentation was most significantly associated with IUIS I (OR 4.21, CI 1.48–11.99) and a molecular diagnosis (OR 2.89, CI 1.23–6.42). Anaemia was the most prominent cytopenia in IUIS VIIb. Class VII did not show marked cytopenia, although lymphocyte count was lower in IUIS VIIa than VIIb (Table S3). The neutrophil-to-lymphocyte ratio (NLR), with an optimal cut-off of 1.8, was associated with IUIS I (OR 2.72 CI 1.01–7.28) and molecular diagnosis, cut-off of 2.1 (OR 2.16, CI 1.19–3.92) with low positive predictive value but high negative predictive value (Table S5).

Death outcome

At the time of last follow-up, 8% (\( n = 20 \)) were lost to follow-up and 11% (\( n = 27 \)) had died. Infections predominated as the cause of death (33%, \( n = 9 \)) followed by organ failure (22%, \( n = 6 \)) with one patient succumbing to respiratory failure secondary to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. Male sex was significantly associated with death (\( p < 0.001 \)). The highest mortality was seen in IUIS I, 21% (\( n = 6 \) of 21 IUIS I cases, none of whom had a bone-marrow transplant), followed by IUIS II 21% (\( n = 7 \) of 33), higher than in those with a molecular diagnosis 17% (13
Extremes of age (2–12 and over 120 months) showed significant association with death (\(p = 0.004\) and \(<0.001\); Table S5). Presentation with cytopenia was significantly associated with death (OR 2.81, CI 1.288–4.158), \(p = 0.005\], namely; severe \(p = 0.003\), and/or recurrent cytopenia \(p < 0.001\) (Table S6). Shorter overall survival was associated with pancytopenia \(p = 0.042\), anaemia \(p < 0.001\) thrombocytopenia \(p < 0.001\], leucopenia \(p = 0.009\] or neutropenia \(p < 0.001\] at presentation (Figure 5 and Table S6).

**FIGURE 2** Distribution of cases according to International Union of Immunological Societies (IUIS) 2019 classification, gender distribution, and clinical presentation. (A) A majority (53%) were classified as a predominantly antibody deficiency (IUISIII), 30% had a confirmed molecular diagnosis. (B) Infections were the most common presentation (86%) and peripheral-blood cytopenia was seen in 62%, 27% had a known family history of IEI (no history of consanguinity). See Table S2 for additional information. Only those IUIS diagnoses with sufficient cases are represented, as well as only the major classifications; subclassifications can be viewed in Table S2. International Union of Immunological Societies (IUIS) I, immunodeficiencies affecting cellular and humoral immunity; IUIS II, combined immunodeficiencies with associated or syndromic features; IUIS III, predominantly antibody deficiencies; IUIS IV, diseases of immune dysregulation; IUIS V, congenital defects of phagocyte number, function or both; IUIS VI, defects in intrinsic and innate immunity; IUIS VII, autoinflammatory disorders; IUIS VIII, complement deficiencies. [Colour figure can be viewed at wileyonlinelibrary.com]

**DISCUSSION**

In this retrospective review of the early haematological findings of IEI in a developing-world setting, cytopenia at presentation was found in 62% of cases. Association between presentation with peripheral-blood cytopenia and a molecular diagnosis of an IEI (OR 2.26 CI 1.42–3.59) and shorter overall survival (OR 2.81, CI 1.288–4.158) was shown.
Cohort characteristics

Almost half were diagnosed within the first five years of life. Presentation with cytopenia was associated with a shorter delay in diagnosis. First-world settings report earlier presentation within the first year of life whilst later presentation is seen in the developing-world setting and reflected this cohort more closely. Patients with IUIS I presented earlier and had a shorter delay in diagnosis; all were cytopenic at presentation namely anaemia, leucopenia and lymphopenia, of which 27% were pancytopenic (Figures 2B, 4D and Table S4). Due to the more severe...
phenotype of IUIS I, classical presentation is typically within the first year of life with severe and/or recurrent bacterial infections as well as cytopenia (commonly lymphopenia, neutropenia and/or thrombocytopenia). In European registries, B-cell disorders are the most common type of immunodeficiency, accounting for approximately 50%–60% of all IEI with most presenting in adulthood, in part due to a less severe phenotype. Reflecting this, the majority (53%) were classified with a predominant antibody deficiency (IUIS III) where presentation with cytopenia showed significant association. As presentation with cytopenia was associated IUIS I and II diagnoses, the predictive value in cohorts with higher consanguinity may be of greater value.

Cytopenia in IEI

Delineation of cytopenia determines whether the cause is increased loss, decreased production or decreased function of cells, namely immune or non-immune pathology. A stepwise laboratory approach focuses on the often lengthy differential diagnosis. Exclusion of infection other than human immunodeficiency virus (HIV) may also be relevant, particularly Epstein–Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, other DNA viruses and bacterial infection. The FBC, DIFF and peripheral-blood film direct the need for haematologic screen (Coombs test, reticulocyte count, indirect bilirubin and haptoglobin), additional useful markers of cell lysis (lactate dehydrogenase, potassium, aspartate transaminase and uric acid) and haematologic studies (iron studies, B12 and folate). Morphological findings of the peripheral-blood film may reveal salient features suggesting an IEI, such as giant azurophilic granules in Chediak–Higashi Syndrome (CHS), hypersegmented neutrophils with pyknotic nuclei and cytoplasmic vacuoles in Warts, Hypogammaglobulinemia, infections and Myelokathexis (WHIM) syndrome and abnormally small platelets in Wiscott–Aldrich syndrome (WAS)/X-linked thrombocytopenia (XLT). The haematologist should be consulted early to guide decisions on flow cytometry and bone-marrow biopsy. Bone-marrow biopsy findings may help synchronise the differential such as myelodysplasia.
in germline GATA2 (GATA binding protein 2) deficiency, myelokathexis in WHIM syndrome, promyelocyte arrest in ELANE (Elastase, neutrophil Expressed) gene mutation and characteristic vacuoles in myeloid precursors in vacuoles, E1 enzyme, X-linked, autoimmune, somatic (VEXAS) syndrome (Table 1). 27,36,37 Pancytopenia, particularly in the child, may highlight underlying IEI such as diseases of immune dysregulation and bone marrow failure syndromes (BMFS). 15,35 One of the main diagnostic challenges in paediatric haematology is the correct diagnosis of patients with pancytopenia and a hypocellular bone marrow. 35 The BMFS often have overlapping morphology and therefore careful past medical, family history and physical examination, looking for syndromic features, is important. 23 35 (Further description of the BMFS can be seen in Table 1).

EuroFlow has developed standardised, accurate and highly sensitive flow cytometric tests for primary immunodeficiency. Although the molecular diagnosis delineates definitive classification in most cases; the cost, often long turnaround time and sometimes difficult interpretation are disadvantages, particularly in the developing setting. Flow cytometry, is already established in most major haematology laboratories, allows quick turnaround time to broadly classify IEI and may focus molecular investigation. 38 The immunologist will direct important immunologic investigation that may be influenced by immunoglobulin replacement or immune suppression which are used in the management of cytopenia, such as quantitative immunoglobulins, vaccine antibody tests, lymphocyte subsets and functional analysis. 2 Reflecting diagnostic yield of molecular testing in both international and local reports ranging from 30%–40%, a pathological variant was confirmed in 30%. 39–41 The molecular diagnosis is a cornerstone of IEI diagnostics, establishing the definitive classification. 4 Molecular analysis is however more expensive and less readily available in some settings and must be guided by clinical phenotype and focused immunological and/or haematological findings. 3,4

Cytopenias in IEI are multifactorial, immune and/or non-immune aetiologies, often multilinage with a chronic or relapsing course and tendency towards treatment refractoriness. 16 Anaemia was associated with an overall shorter overall survival in this cohort (Figure 5). Only one patient, with a complement deficiency, presented with autoimmune haemolytic anaemia (AIHA). Haemolysis in complement deficiency or myelosuppression from immune dysregulation or secondary infection/malignancy may also be contributory. 4 Anaemia was seen in IUIS V and VI, of which Chronic Granulomatous Disease (CGD) and Mendelian Susceptibility to Mycobacterial Disease (MSMD) fall into these categories respectively. Anaemia in CGD is probably attributable to decreased iron absorption or increased losses as a result of colitis as well as chronic disease. 45 Both CGD and MSMD carry an increased risk of tuberculosis (TB) infection. TB is associated with anaemia which is usually anaemia of chronic disease. Interestingly, anaemic patients may be at increased risk of TB infection. 46

Lymphopenia in association with severe infections in infancy was described in early severe combined immunodeficiency (SCID) case reports and as a result has long been an early warning sign of immunodeficiency, incorporated into scoring systems for early identification of IEI. Severe (<3 × 10⁹/l) or persistent lymphopenia in an infant with a suggestive history should prompt immediate immunological investigation for IEI. 28 More than half (62%) of the cohort were lymphopenic at presentation. A raised NLR was predictive of IUIS I and a molecular diagnosis of an IEI, likely reflecting relative lymphopenia (Table S5). B- and T-cell immunodeficiencies commonly present with lymphopenia due to intrinsic cell defect, abnormal maturation and function and altered cytokine secretion (Figure 1D). 2,47,48 Closer investigation into the lymphocyte count with lymphocyte subsets will help focus the differential diagnosis and immunological investigation. 10,16

Neutropenia is common in IEI, an absolute neutrophil count less than 1.5 × 10⁹/l in patients over one year old or chronic neutropenia (lasting >3 months) a likely caveat. 27,49 The haematologists’ early involvement is important as the peripheral-blood and bone-marrow findings along with careful clinical examination can guide the often lengthy differential (Table 1). 27 Non-immune causes such as severe infection common across all IEI groups, particularly in deficiencies of phagocyte number or function or bone-marrow infiltration, should also be considered (Figure 1C). The direct involvement of Bruton tyrosine kinase (BTK) in neutrophil development is unclear, but is thought to contribute to neutrophil differentiation resulting in neutropenia, likely explaining why neutropenia was predictive of hypogammaglobulinaemia in this cohort. 27,49 No clear association was seen with defects of phagocyte number or function to further divulge the association, likely attributable to the low number of cases in the cohort. One case however, with a confirmed ELANE mutation, did present with classical cyclic neutropenia.

Immune thrombocytopenic purpura (ITP) is common in IEI and may be associated with other immune cytopenias such as Evans syndrome (ES) (Figure 1B, Table 1). 2,15,44 In a child, ES should prompt investigation for a possible monogenic defect. 16 In this cohort thrombocytopenia was predictive of combined immunodeficiency (CID) with associated syndromic features (IUIS IIa) which includes WAS/XLT, the classical thrombocytopenic IEI with abnormally small platelets.

Inborn errors of immunity carry an increased risk for developing malignancy. 17,21–23,30 Haematological malignancy was seen in three cases, all with B-cell lymphoma. The development of malignant neoplasm is complex and related to the inherent impaired immunity, tumour immune...
**Haematological signs that should prompt further investigation for IEI**

| Pathophysiological mechanism | Associated Inborn Error of Immunity | Common haematological findings |
|-----------------------------|-------------------------------------|--------------------------------|
| Autoimmune-mediated cytopenia in IEI | CID, CVID | Immune cytopenia: potentially life threatening: AIHA and ITP, autoimmune neutropenia, Evans syndrome² |
|  | ALPS, LRBA | Non-Immune cytopenia: thrombocytopenia, Lymphoproliferative disorders associated with viral infections² | |
|  | X-linked agammaglobulinemia (XLA) | Immune cytopenia, neutropenia (up to 10%–26%), decreased bone-marrow precursor, decreased myeloid maturation (BTK-related signal transduction)² |
|  | SCID | Immune cytopenia, lymphopenia, eosinophilia, lymphocytosis in Omenn syndrome²,¹⁷ |
|  | CID, WAS, 22q11 deletion (DiGeorge Syndrome) | Immune cytopenia (refractory cytopenia of childhood), non-immune cytopenia — lymphopenia, lymphoproliferation, microthrombocytopenia [low mean platelet volume (MPV) in WAS] |
|  | PI3KD | Lymphoproliferative disorders associated with viral infections |
| Immune dysregulation underlying cytopenia in IEI | IPEX and IPEX-like APECED | Immune cytopenia, lymphoproliferative disorders associated with viral infections, eosinophilia, lymphocytosis |
|  | Familial haemophagocytic lymphohistiocytosis 1–5 (FHL) | Hyperferritinaemia, splenomegaly, cytopenias, haemophagocytosis |
|  | Hermansky–Pudlak syndrome (HPS) | Bleeding diathesis normal platelet count and size: impaired secondary aggregation in platelet aggregometry, neutropenia³⁵ |
|  | Chediak–Higashi Syndrome (CHS) | Normal platelet count and size, large azurophilic granules in lysosomes (neutrophils, eosinophils), neutrophilia common, defective granulocyte mobilisation from bone marrow, increased turnover due to increased destruction²⁷ |
|  | WHIM syndrome | Myelokathexis: retention of neutrophils in the bone marrow due to increased CXCL12 responsiveness thus peripheral neutropenia, hypercellular marrow⁴⁹ |
|  | X-linked lymphoproliferative disease (XLP), IL-2-inducible T-cell kinase (ITK) deficiency, ALPS | Immune cytopenia — Evans syndrome, lymphoproliferative disorders associated with viral infections²,⁴⁴ |
| Phagocyte disorders | Schwachman–Diamond syndrome | Neutrophenia, thrombocytopenia and anaemia may be severe, pancytopenia, maturation arrest myelopoiesis, hypocellular marrow, increased risk leukaemia³⁸,⁴⁵ |
|  | Elastase deficiency (ELANE mutation) | Cyclic neutropenia, promyelocytic arrest (exclude acute promyelocytic leukaemia)²⁷ |
|  | GATA2 deficiency | Neutropenia common, neutrophil maturation preserved, monocyte myelodysplasia, atypical megakaryocytes, increased risk acute myeloid leukaemia²⁷ |
|  | Chronic granulomatous disease (CGD), X-linked neutropenia | Neutrophilia, abnormal neutrophil function (commonly demonstrated with neutrophil oxidative burst assay), normal neutrophil morphology on peripheral blood film and/or bone marrow aspirate |
| Bone-marrow failure in IEI | Schimke syndrome | Cytopenias, often pancytopenia. |
|  | ALPS and FHL | |
|  | Dyskeratosis congenita (DKC) | Pancytopenia, increased risk: bone-marrow failure, myelodysplasia and acute myeloid leukaemia |
|  | Cartilage hair hypoplasia (CHH) | Neutropenia, lymphopenia |
|  | Reticular dysgenesis | Absence of neutrophils, bone-marrow failure |

(Continues)
surveillance as well as viral infections such as EBV.\textsuperscript{50,51} The type of malignancy is closely associated to the underlying type of IEI, for example non-Hodgkin lymphoma frequent in common variable immunodeficiency (CVID) and acute myeloid leukaemia in WHIM syndrome.\textsuperscript{23,50–52} Chronic infections, such as EBV, establish a persistent inflammatory stage creating tissue damage with subsequent malignant transformation.\textsuperscript{52} Immunologists need to diagnose the malignancy and haematologists/oncologists the underlying immunodeficiency early to optimise management and outcome.\textsuperscript{50}

Despite significant advances in diagnosis and treatment, many IEI patients still suffer unnecessary premature death.\textsuperscript{14,54} X-linked IEI often display a more severe phenotype, males having a shorter overall survival, as reflected in this cohort. Severe and recurrent cytopenias were most significantly associated with death. Presentation with pancytopenia, anaemia, thrombocytopenia and leucopenia had a shorter overall survival (Table S6). This may reflect more advanced disease at presentation or severe phenotype that often has a shorter overall survival, especially with delayed intervention.

**Limitations**

The retrospective nature of the study reflects only a window of time, at presentation, and patients may have developed immune cytopenias or malignancy over their disease course. Although greatest effort was made to delineate the nature of cytopenia based on laboratory history, clear aetiologies were not defined. Infectious manifestations are more widely known, and as case identification relied heavily on clinician identification, entry onto the SAPIDR may have been weighted toward infectious manifestations. The review of the literature has aimed to compensate for these limitations and attempted to provide an overview of both immune and non-immune mechanisms of cytopenia in IEI. Such limitations highlight the need for more prospective reviews with inclusion of age-matched controls.

**CONCLUSION**

A high index of suspicion is imperative for early identification of patients with IEI. Both infectious and non-infectious manifestations must be considered.\textsuperscript{10,12,13} Peripheral-blood cytopenia that is severe, persistent, unusual and/or recurrent (SPUR) should prompt further investigation for IEI in both children and adults. The cost-effective and universally available FBC and leucocyte differential could facilitate earlier case identification, serving as an adjunct to more costly definitive investigation. Although inconclusive in some, the molecular diagnosis is a cornerstone of IEI diagnostics, establishing definitive classification.

**Recommendations**

1. The FBC with leucocyte differential and peripheral-blood film is widely available and cheap. It should always form part of first-line investigation in suspected IEI.
2. Severe, persistent, unusual and/or recurrent (SPUR) cytopenia in the absence of secondary immunodeficiency should prompt further definitive investigation for IEI in children and adults.
3. Prospective review of patients presenting with SPUR cytopenia(s) prompting investigation for possible IEI with application of flow cytometry and molecular diagnostics to confirm diagnosis would provide further insight.

**AUTHOR CONTRIBUTIONS**

H. M. Cornelissen, Z. C. Chapanduka and E. Musekwa conceptualised and designed the study. H. M. Cornelissen, Z. C. Chapanduka, E. Musekwa and R. H. Glashoff wrote the draft protocol and manuscript. H. M. Cornelissen facilitated data collection, statistical review, primary manuscript composition and the creation of figures with expert review and contribution from Z. C. Chapanduka, E. Musekwa, R. H. Glashoff, M. Esser, D. R. Abraham and M. Zunza. M. Zunza conducted the statistical analysis and contributed to manuscript review. M. Esser and D. R. Abraham contributed to
clinical history, manuscript review and expert clinical opinion.

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CONFLICT OF INTEREST

None to declare.

DATA AVAILABILITY STATEMENT

Extensive supplementary data has been submitted. Additional data will be made available on request.

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