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Review

Low flow: Selecting a limited flow cytometry panel where resources are constrained

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ABSTRACT

Diagnosis and treatment of patients with haematological malignancies (HM) is hampered by access to pathology services in resource-limited settings (RLS). Internationally accepted guidelines and diagnostic criteria for HM require access to sophisticated analysis including comprehensive flow cytometry (FCM) for minimum essential diagnosis and treatment, which is technically challenging in RLS. This review will define these shortcomings and examine the use of limited FCM panels in RLS.

While a consensus guideline exists for a limited chronic lymphocytic leukaemia (CLL) FCM panel, this has yet to be validated in a large cohort. Currently, there are no consensus-based and resource-stratified diagnostic protocols defining limited FCM panels for the diagnosis of acute leukaemia where resources are limited.

There is an unmet need for such guidelines, supported by evidence, for the diagnosis of the most common HM. This systematic review defines consensus-based limited FCM panels from the literature that may be used in the interim.

1. Introduction

Health care priorities in low- and middle-income countries (LMICs) are focused on prevention and treatment of communicable disease. The success of these programs has shifted the disease burden to non-communicable diseases such as cancer [1–3]. Haematological malignancies (HM) including leukaemia, lymphoma and multiple myeloma account for 6.2 % of all new cancer diagnoses and 6.9 % of cancer deaths globally [4]. Accurate diagnosis of HM is critical to providing adequate treatment [5]. In high-income countries where cancer is a leading cause of morbidity and mortality, there is an abundance of research, literature, and high-quality health data. Where resources are constrained in LMICs, however, studies are rare, data quality is low, and the interpretation of this data is unclear. Although much of the data published around resource limitations arises from LMICs, the problem extends to all areas where health care provision is constrained due to poor resources. These areas are not exclusively within LMICs and are termed resource-limited settings (RLS), and are defined by their limited capability to provide care for life-threatening illnesses [6]. RLS also exist within high-income countries, and inferior patient outcomes in these areas are well documented [7,8]. Cancers, including HM, are diagnosed later and at a more advanced stage of disease in these settings. Patients are also younger and malignant disease rates are reportedly lower, even when incidence or mortality rates are age-standardised [2,4,9,10]. It remains unclear from the literature whether the incidence of cancer in RLS is reportedly lower due to the lack of industrialisation [4], genetic variation [11] or the significant lack of access to cancer diagnostic services [12]. Though under-diagnosis likely plays a significant role, clarifying the reasons for lower HM prevalence in RLS requires more extensive research [12].

1.1. Current gold-standard diagnostics

Diagnostic analyses for HM range from inexpensive blood film morphology to very sophisticated and complex molecular analyses. Blood and bone marrow microscopy alone does not provide sufficient evidence for minimum essential treatment standards for many HM, despite this being the only diagnostic tool available in most RLS [5,13–15]. Guidelines for the diagnosis of HM are well established and widely available [16,17]. These guidelines rapidly evolve over time as more accurate diagnostic tests become available, and the requirement for access to expensive and complex testing has increased [18]. Unfortunately, consensus diagnostic guidelines, such as the world health

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organisation (WHO) classification of haematolymphoid neoplasms and the EuroFlow™ diagnostic algorithm require a level of access to diagnostic tests that most physicians in RLS do not have [16,17,19]. While the newest WHO guidelines, WHO-HAEM5, have divided diagnostic criteria into 'essential' and 'desired', the minimal recommendations often include FCM and molecular testing [16].

1.2. Challenges in resource-limited settings

Outcomes for patients diagnosed with HM in LMICs are inferior to those in high-income countries [19]. Though the underlying reasons for this are multifaceted, delayed diagnosis, lack of accuracy in diagnosis and reduced access to effective therapies all play a significant role [4]. This is further compounded in RLS due to low health literacy, symptom overlap with non-communicable diseases and a lack of appropriate diagnostic services. Clinicians in LMICs are often attempting to use guidelines developed for high-income countries that are inappropriate where resources are constrained, or outdated recommendations, and this has an impact on patient outcomes [20]. Accurate and timely diagnosis is essential for adequate treatment. Therapies for HM are becoming less toxic, more orally deliverable and more accessible in RLS, as evidenced by Chronic Myeloid Leukaemia (CML) [21]. Accurate diagnostic testing, however, continues to lag behind the availability of treatment options, thus patient outcomes could be greatly improved by facilitating access to appropriate diagnostic services.

1.3. Flow cytometry barriers

1.3.1. Diagnosis of haematological malignancies

The fundamental diagnostic test for HM is the microscopic assessment of a stained peripheral blood or bone marrow smear. Morphology assessment has limitations, with diagnostic errors estimated to be around 20–30 % when compared with advanced testing and comprehensive FCM [22]. Cytochemical staining for cell features such as myeloperoxidase granules improves the accuracy of microscopic classification. Although widely accessible, microscopy and cytochemistry often do not provide adequate diagnostic accuracy, particularly for lymphoid malignancies [5,23,24]. Subtle morphological differences can have an enormous impact on treatment regimens and patient outcomes even where treatment options are limited [5]. Immunophenotyping using FCM is recommended, and often regarded as essential, for diagnosis and treatment [14,15]. Identification of the cell of origin and maturation stage of malignant blood cells using microscopy and FCM allows more specific disease classification [16,17]. A small number of HM require molecular or cytogenetic analysis for diagnosis, however FCM usually allows a sufficient diagnostic level for treatment stratification in RLS [5,14–17].

1.3.2. Necessity for flow cytometry in resource-limited settings

To maximise clinical efficacy and efficiency, disease diagnosis should be predicated on available treatment options. Morphological diagnosis of HM provides a reasonable level of accuracy for AML as many of the FAB subtypes are visually distinct. Treatment options for AML in RLS often do not require the deep level of lineage classification that is achievable by flow cytometry [5,14–16]. Where only basic resources are available, mandatory or essential FCM is recommended for lineage determination of ALL and acute leukaemias of undetermined or ambiguous lineage [5,14–16]. Whilst the greatest benefit would be achieved for classification of acute leukaemias, implementation of FCM in RLS would also allow immunophenotyping for non-Hodgkin lymphoma (NHL) and other HM. Additionally, there are benefits to identify potential treatment targets, such as CD20 for rituximab therapy, and this is reflected in some basic panel recommendations [15,25,26]. Treatment regimens for these entities vary significantly, and limited FCM access provides a greater benefit for these disease classes and subtypes as defined in WHO-HAEM5 32.

1.3.3. Technical and logistical limitations

While FCM offers an optimal diagnostic option in RLS, there are significant technical limitations. Diagnostic multiparametric FCM has become increasingly complex to perform, with rapidly evolving instrument capabilities and availability of antibody-fluorochrome conjugates [18]. Initially using only 4 different antibody-fluorochrome conjugates (4 colour) in the 1990's, comprehensive clinical FCM has progressed to 6, 8, 10 and now 12 colour protocols in routine use. With this increase, instrument setup and protocol validation has become exponentially more difficult due to fluorochrome compensation and data interpretation issues [27,28]. Substantial institutional variation in sample quality, hardware, instrument settings, reagents, software, gating strategies, scatterplot interpretation, resource access and operator skill make interlaboratory comparisons difficult. It is widely accepted that there is variability amongst testing sites and that standardisation is a challenge [27–30]. While these challenges are faced by all health services, restriction of resources exacerbates the effects of technical complexity. The extent of the disparity between haematology diagnostic practices in areas with high resources compared to those with limited resources is unknown [31–33]. Agreement on a limited panel of markers with demonstrated diagnostic accuracy that is cost-effective and logistically feasible would improve HM diagnosis in RLS [16,17,30].

1.3.4. Limited flow cytometry panels

Simplification of FCM using limited panels of 10–20 antibodies and 4–6 fluorochromes approximates the implementation phase of clinical flow cytometry, where reduced panel complexity still provided diagnostic improvements over morphology. This approach has the potential to reduce the training burden, instrument compensation, troubleshooting, antibody titrations and reagent wastage when compared to more comprehensive FCM. EuroFlow™ comprehensive flow cytometric panels are well established, validated and evidence-based [30]. The WHO-HAEM5 guidelines 'desired' FCM has a high degree of correlation with EuroFlow™, and together often recommend a catalogue of more than 30 antibody-fluorochrome conjugates and 8 colour FCM for classification of HM. Acquisition and appropriate storage of the extensive conjugate library required for comprehensive FCM can be difficult and cost-prohibitive in RLS [19]. Studies have explored limited panels of 10–20 antibodies to reduce the costs for FCM [13–15,26,33–37]. In high-income countries, comprehensive FCM is essential to assess minimal residual disease, however this is often not a priority for lower income countries due to available treatment options. Limited FCM panels have demonstrated some success and are currently used in many RLS, however studies exploring limited panels are rare and will be examined in this review.

1.4. Objectives

This systematic review will evaluate studies and guidelines recommending diagnostic protocols for HM, specifically where resources are constrained. Flow cytometry is required for the most basic diagnosis of HM [5], therefore the primary aim of this review is to define a consensus-based limited panel comprising recommended antibodies from the literature. As part of this objective, a meta-analysis of limited FCM panel antibodies and their use in RLS will be conducted. The second aim is to explore and describe currently available HM diagnostics in RLS and compare these with gold-standard diagnostic practices. Finally, the barriers to accessing diagnostic services in RLS will be defined to understand the economic, logistic, and technical limitations in these areas. Potential pathways to improving diagnostic accuracy in these areas will also be recommended.

2. Methods

A systematic review of the literature was conducted to assess the current protocols in use for diagnosing HM in RLS. The reporting of this

systematic review was guided by the standards of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Statement [38].

2.1. Literature search

Search criteria were based on Medline Medical Subject Heading (MeSH) terms to include all terms related to Haematological Neoplasm, Flow Cytometry, and Resource-limited Settings. Many LMICs and RLS did not identify as such, so all countries defined as 'LMIC' calculated using the World Bank Atlas method in October 2024 were included individually in the alternate search terms for RLS. The low number of published studies necessitated search terms that were significantly expanded to capture any relevant literature. The expanded set of terms were used to search Web of Science, Medline: Ovid, Scopus and Cinahl. Additional articles were found in the grey literature, including google scholar and relevant citations from included publications (Fig. 1). Initial search strategies excluded non-English language and animal studies. The timeframe was restricted to 1 January 2005 until the final search was conducted on 10 October 2024. Prior to 2005, FCM panels in routine use could mostly be defined as 'limited' (<20 antibodies) and this was verified by a search of earlier articles.

2.2. Eligibility criteria

This review aimed to explore barriers to testing and to identify diagnostic shortfalls when compared to gold-standard testing, therefore publications defining current diagnostic practices for HM in RLS were selected. This included a small number of studies describing novel diagnostic tests for HM in RLS.

2.3. Study selection

Inclusion criteria for studies reviewed were: (1) Country was defined as LMIC or study identified as resource-limited, (2) A limited FCM panel (<20 antibodies) was defined with specific antibody recommendations, (3) Studies included subjects with haematological malignancies, (4) Diagnosis of malignancy was compared to at least one other method, (5) Access to FCM was quantified, (6) Guidelines for diagnosis of haematological malignancies that may be used in RLS. After initial review, published results were included if there was a defined population and study period, the study included observations of peripheral blood or bone marrow, the methods for diagnostic evaluation were stated, and the method for HM classification was outlined.

2.4. Exclusion criteria

Some studies used comprehensive FCM techniques through collaboration with high-income countries. These studies were excluded if there were more than 20 antibodies in the FCM panel for acute leukaemia, as this was not sufficiently resource-constrained for the purposes of this review. Haematological malignancies such as myeloproliferative neoplasms, myelodysplastic disorders and Hodgkin lymphoma were excluded as FCM is not essential for their diagnosis, as defined by WHO-HAEM5 [39]. Single case studies were also excluded.

2.5. Data extraction and analysis

Data were collected from each article including authors, year, country, study duration, number of participants, study design or guideline, haematological malignancy focus and classification level, use and definition of limited FCM panel, quantitation of FCM access, and study findings. A meta-analysis was conducted of limited FCM panel components, accuracy of diagnosis when compared to existing diagnostic practices and access to FCM in RLS.

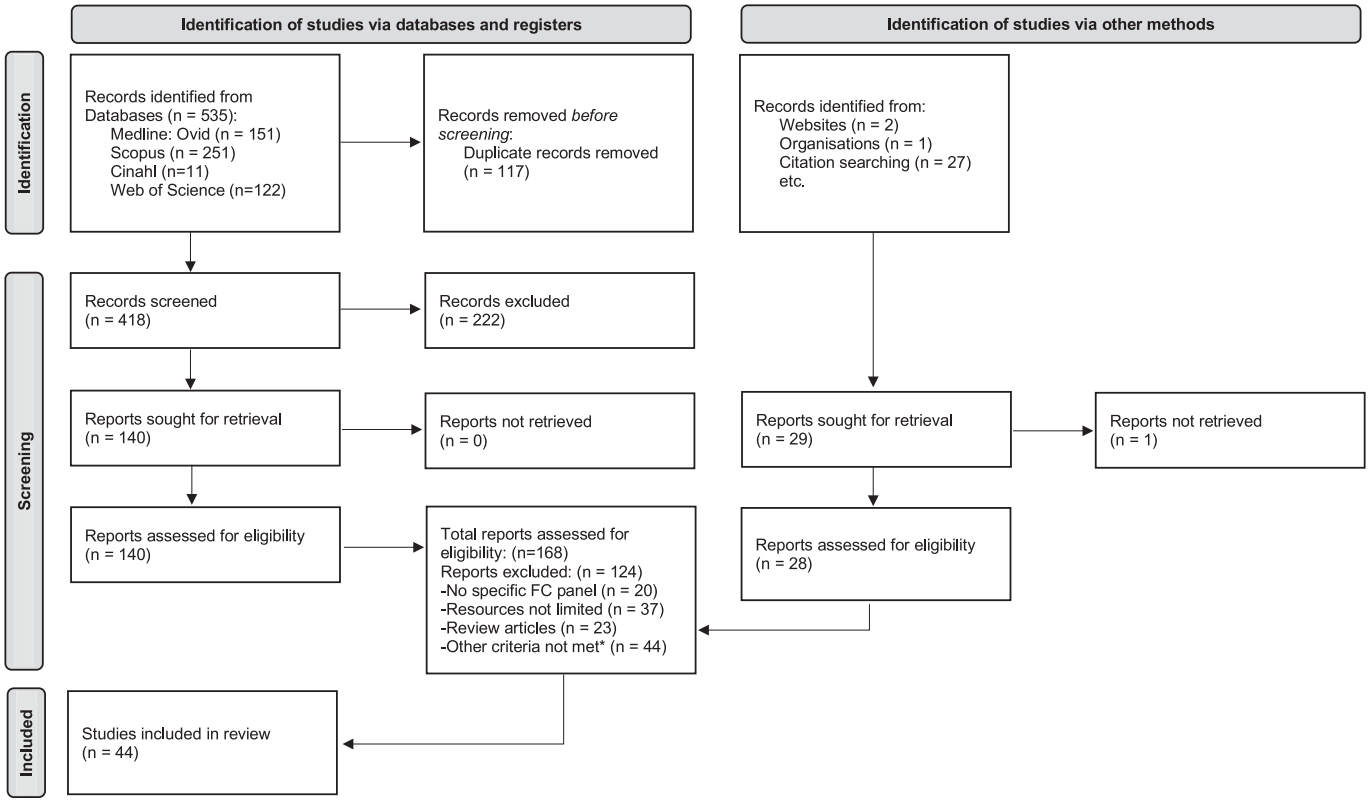

Fig. 1. Flow diagram of literature search and selection criteria [40].

3. Results

3.1. Study selection for meta-analysis

This systematic review included published diagnostic guidelines, cross-sectional and cohort studies, and articles detailing novel diagnostic procedures suitable for RLS. All 44 papers selected were from areas identified as resource-limited, countries defined as LMIC in October 2024, or were guidelines used for diagnosis of HM in these areas (Fig. 1). Countries included in this review are Brazil, Guatemala, Sudan, Ethiopia, Nigeria, Kenya, rural Uganda, Iraq, Iran, India, Bangladesh, Vietnam, Philippines, Myanmar and Indonesia. Resource access is not uniform across these low- and middle- income countries, and some included regions may not have significant resource restriction, which is a limitation of this review. 18 articles provided limited panel FCM information for acute and chronic leukaemia. Included in these were three resource-stratified guidelines for the use of FCM in acute leukaemias where resources were limited [5,13,15] and an international consensus CLL harmonisation guideline [37]. Seven guidelines for diagnosis of HM that were not limited FCM or sufficiently resource-stratified met the inclusion criteria, as they are often used in RLS [16,17,19,20,30,40,41]. The remaining 4 studies outlined novel diagnostic testing for HM in these areas.

3.2. Study quality

3.2.1. Selections bias

The CASP (critical appraisal skills program) for cohort studies tool was used as a basis to assess the quality of the studies included in this review. It was found that most of the studies did not provide sufficient detail for a comprehensive analysis and would be excluded on this basis if more studies were available. Of the 23 studies that included participant numbers (Table 1), 13 performed FCM as part of routine studies, 4 did not routinely perform diagnostic FCM [33,35,42,43] and 5 studies that performed FCM did not stipulate whether testing was carried out as a part of routine patient investigations or was limited to the published study [24,26,44–46]. Inclusion criteria were also undefined or unclear in many studies, so there was likely significant bias toward patients who could afford treatment or had complete and accessible records. Only 8 studies were assessed to have no bias, 7 identified a bias [34,42,43,47–50] and the remaining 8 studies did not provide sufficient detail to determine bias, however these were included in the review due to the paucity of publications [11,22,26,33,36,44,46,51].

3.2.2. Study participants

The number of participants recruited was examined along with the rate of recruitment. There was a high degree of variation, with 3.1 patients recruited per year for a CLL study in Nigeria and 1253 in a HM

Table 1
Overview of 23 included clinical studies.

Author	Year	Study design and duration	Target population	Country	HDI	Sample size	Median age	Flow cytometry panel	Highest classification level (routine)
Abdelgadar et al. [42]	2018	Prospective, 0.4 years	CLL	Sudan	0.516	99	63	Limited chronic	CLL
Ahmad et al. [47]	2024	Prospective, 1 year	ALL, AML	India	0.644	**57**	NR	NR	AML, ALL
Al-Sharifi et al. [36]	2016	Prospective, 2 years	HM	Iraq	0.673	79	NR	Limited acute	B/T ALL, AML, BAL
Antillon et al. [25]	2016	Prospective, 7 years	ALL <18yo	Guatemala	0.629	787	NR	Limited acute	B/T ALL
Dayton et al. [48]	2024	Prospective, 2 years	T-LGL	Vietnam	0.726	53	NR	Limited T-cell only	T-LGLL
Gupta et al. [26]	2016	Prospective, 2 years	HM	India	0.644	82	31	Limited acute	B/T ALL, AML(FAB)
Hossain et al. [49]	2014	Retrospective, 4 years	HM	Bangladesh	0.67	5013	42	No flow cytometry	B/T ALL, AML(FAB)
Korubo et al. [50]	2021	Retrospective, 15 years	CLL	Nigeria	0.548	46	55	Limited chronic	CLL
Madu et al. [52]	2019	Retrospective, 8 years	CLL	South Nigeria	0.548	97	59	Limited chronic	CLL
Medalla et al. [34]	2021	Retrospective, 3.3 years	ALL	Philippines	0.71	268	NR	Limited acute	AML, ALL, AUL
Mehrvar et al. [43]	2015	Retrospective, 4 years	AML <15yo	Iran	0.78	104	6.5	NR	AML(FAB)
Mulwu-Babu et al. [44]	2013	Prospective, 0.5 years	CLL	Kenya	0.598	49	62.1	Limited chronic	CLL
Navarrete et al. [53]	2014	Prospective, 4 years	ALL <18yo	Central America	0.7	1313	NR	Limited acute	B/T ALL
Okoye et al. [22]	2020	Retrospective, 20 years	HM	Nigeria	0.548	129	46	FCM restricted, panel NR	B/T ALL, BAL, MPO neg AML
Rawstron et al. [35]	2017	Prospective, 0.9 years	CLL	Rural Uganda	0.55	302	NR	Limited chronic	AML, ALL
Salaam et al. [24]	2019	Prospective, 0.5 years	HM	India	0.644	56	NR	NR	AML, ALL, CML, CLL
Sengar et al. [32]	2009	Prospective, 1 year	HM	New Delhi	0.644	100	18	Limited acute	AML, ALL
Shwe et al. (90)	2019	Retrospective, 2 years	HM	Myanmar	0.608	295	NR	NR	B/T ALL
Singh et al. [46]	2016	Prospective, duration NR	HM	India	0.644	200	28	Limited acute	B/T ALL, AML(FAB), MPAL
Sukrisman et al. [11]	2022	Prospective, 6 years	CLL	Indonesia	0.713	38	59.5	Limited chronic	CLL
Supriyadi et al. [67]	2011	Prospective, 4 years	ALL <15yo	Indonesia	0.713	541	NR	Limited acute	B/T ALL, AML(FAB), MPAL
Tegegen et al. [33]	2021	Prospective, 2 years	HM	Ethiopia	0.492	11**77**	NR	Limited chronic and acute	AML, ALL
Varghese et al. [54]	2018	Retrospective, 5 years	ALL <15yo	India	0.644	**75**	4	NR	B/T ALL

Abbreviations: HDI = Human Development Index, Maturation stage classification – AML(FAB) = French-American-British morphological classification AML M0–7. NR = Not reported.

study in Bangladesh [49,51]. The interquartile range of the number of patients recruited per year in these studies is 122 (89–148). Although an analysis of recruitment vs population was not able to be conducted, it is clear for many studies that recruitment was quite low given the population density.

3.2.3. Qualitative studies

A small number of qualitative studies were included in this review, including 2 surveys [19,20] and a review article [52], as they offered unique perspectives and data on the use of diagnostic guidelines, distribution of global resources, availability of FCM and underdiagnosis of HM.

3.2.4. Accuracy of flow cytometry

The accuracy of FCM itself was not assessed by any studies in this systematic review. Flow cytometry results were assumed to be the gold-standard to which other diagnostic tests are compared due to a lack of more advanced or corroborative tasting [26]. It was rare for an included study to acknowledge the limitations of FCM, the difficulties in standardising results or the negative impacts of the many confounding factors. Only one study compared the accuracy of limited FCM to comprehensive FCM [46], which is the most relevant correlation analysis due to the inclusion of gold-standard testing. Another study compared two different limited panels [26] and 4 studies compared morphology to limited FCM (Fig. 3). No studies were found that compared the accuracy of morphology to both limited and comprehensive FCM.

3.3. Published diagnostic practices

Both prospective and retrospective clinical studies outlined current routine diagnostics for HM in RLS (Table 1). These studies covered a broad range of LMICs, 76 % of which were low-income countries. Although articles with a treatment focus were largely excluded, studies did have relevant details on diagnosis so were included [25,50,53,54]. While only 14 of the 23 studies had a diagnostic focus, 15 studies provided specific FCM panels (Table 1), and 4 of these had sufficient information for a small meta-analysis (Fig. 2).

3.4. Classification of haematological malignancies

Due to higher disease prevalence, most studies focused on acute leukaemia and NHL, including chronic lymphocytic leukaemia (CLL). Acute lymphoblastic leukaemia (ALL) is the most common paediatric cancer, and 5 studies focussed on this HM. Access to FCM affected the depth of classification level, with variable classification levels achieved (Table 1).

3.5. Guidelines in resource-limited settings

An ideal diagnostic panel for FCM in an RLS would be a resource-stratified, international consensus guideline validated with patient data. While an international consensus panel has been proposed for CLL [37], it is yet to be validated with patient data in a large cohort.

There is disagreement between professional groups regarding a harmonised guideline for acute leukaemia. Ten guidelines on the diagnosis of HM were included for analysis [5,13–17,30,37,40,41]. These guidelines provided specific FCM panel recommendations or information highly relevant to general diagnosis of HM.

3.5.1. Resource-stratified guidelines

This systematic review found only four resource-stratified guidelines published for limited FCM availability. These include an ALL-specific consensus guideline from the 2013 Asian Oncology Summit [15], the National Comprehensive Cancer Network (NCCN) in the U.S. for a range of HM [5] and the Inter-Laboratory Comparison Program (ILCP) guidelines for hematolymphoid neoplasms in India [13]. Lastly, there is a four-colour acute leukaemia guideline from a Brazilian FCM group (GBCFLUX) [14]. This document is unique in that it recommends a screening panel, followed by specific disease-specific follow-up panels in the style of EuroFlow™. The inclusion of a screening panel makes the GBCFLUX recommendations less useful in a RLS, as resources are not usually available for sample screening. The antibody panels

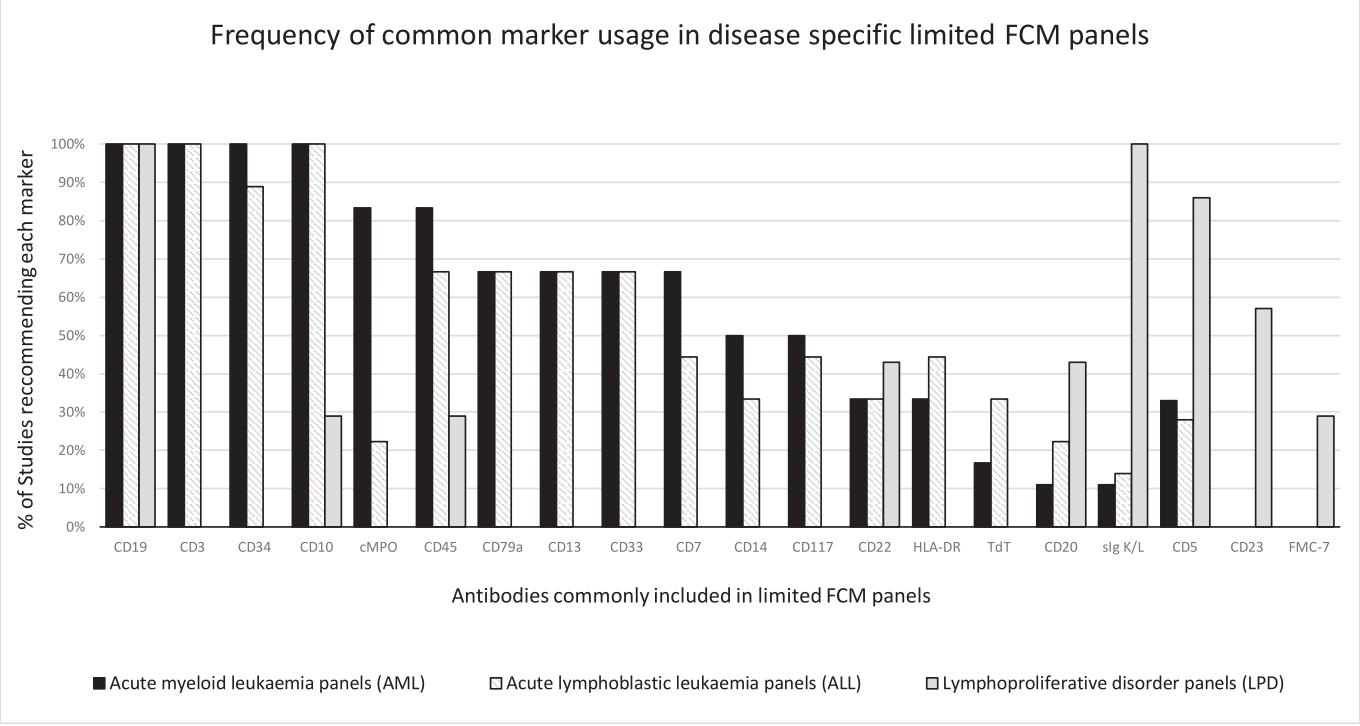

Fig. 2. Frequency of CD marker recommendation by studies that defined a limited FCM panel.

recommended are tiered according to necessity and include the lowest levels of resource access, however this makes it difficult to compare with other panels that describe a single limited panel, so the GBCFLUX recommendations are excluded from the meta-analysis. These region-specific guidelines had significant discrepancies, such as the inclusion of fundamental myeloid antibodies and maturation markers in limited FCM panels such as CD13, CD33, CD117 and CD34.

3.5.2. Inclusion of consensus international guidelines

Three current international consensus guidelines were found, with publications from the WHO and EuroFlow™ [16,17,30]. These guidelines are primarily designed without resource-stratification and even when resources are considered, a moderate level of access to FCM for 'essential' diagnostics is presumed. Bethesda and EGIL international consensus guidelines have been superseded but were included in this review as they are currently still used in some RLS due to their simplicity [40,41].

3.6. International partnerships

Due to the complexity of FCM, diagnostic collaborations between LMICs and expert laboratories in higher-income areas via telehealth or telemedicine were likely occurring in the studies in this review. However, due to a lack of detail in many of the publications, the extent of this involvement was unable to be quantified. It was often unclear where the FCM was being performed, and if there was participation of partner organisations or expert laboratories to assist with disease diagnosis. Studies performing comprehensive FCM with more than 20 antibodies in their panel were excluded, and only one included study had access to comprehensive FCM and compared it to a limited panel [46]. A study from Indonesia collaborated with the Dutch Cancer Society, but the ongoing nature of this collaboration are not detailed [55].

Defining the nature of collaborations between high and low-middle income areas was difficult from this review, but such collaborations have been described more generally in the literature. Often termed as 'twinning', these arrangements are suggested as a pathway to improving diagnostics for cancer, including HM, in RLS [56–59]. The lack of appropriate pathology services in LMIC is a complex and multifactorial issue, and greater success and improvements can be seen when sustained collaborations occur [60,61]. With the growing availability of telehealth, this may be a viable option for RLS, but there are inherent issues such as reliable access to technology, human resources and maintenance of partnerships which are difficult to overcome [60]. Additionally, degradation of flow cytometry samples over time and inherent cost and logistical difficulties of transporting biological samples from LMIC to sophisticated HIC laboratories able to provide gold standard testing means that such partnerships may be more beneficial in an advisory capacity.

4. Defining a consensus-based limited flow cytometry panel

Of the 23 clinical patient studies included in this review, 9 studies provided specific limited FCM panels for acute leukaemia diagnosis and 7 studies outlined a limited panel for lymphoproliferative disorders (Table 2). Each limited panel was found to be unique when the 21 most frequently recommended antibodies were compared across the 16 studies, although some consistencies were noted (Fig. 2). CD19, CD3 and CD10 were included in all acute leukaemia limited panels, and all lymphoproliferative disorder limited panels included CD19, sIg Kappa and sIg Lambda (Fig. 2). Aside from these 5 markers, significant variability across panels was seen, suggesting a need for standardisation.

4.1. Acute Leukaemia

Limited FCM panels in use for the diagnosis of acute leukaemia were described in 11 studies. Resource-stratified guidelines were also included for comparison [13–15] and two studies that cited use of these guidelines were excluded due to redundancy [23,62]. Overall, there were 12 unique limited FCM panels included in the meta-analysis for acute leukaemia, comprising 9 studies and 3 guidelines (Table 2). The EuroFlow™ acute leukaemia orientation tube (ALOT) was included for comparison, but this was not designed as a stand-alone limited FCM panel. The WHO essential IVD panel was also not included due to a lack of supporting data and guidelines for use [63].

4.1.1. Limited flow cytometry panel for acute leukaemia

Of the FCM panels in this review described for acute leukaemia, both the mean and median number of antibodies included in a limited panel were 10. This is a logistically feasible number of markers for a consensus-based limited panel, offering a compromise between cost, complexity, and utility. To define a 10-antibody limited FCM panel for acute leukaemia diagnosis, publications were analysed for recommended CD marker inclusion. Using the frequency of antibody inclusion within the selected studies and guidelines, the top ten most used CD

Table 2
Proposed limited FCM antibody panels for acute leukaemia from included publications. S = surface antibody staining method, c = cytoplasmic antibody staining method, ab. = antibody-fluorochrome conjugates

Panels with < 20 ab.	Total ab.	CD19	CD3	CD34	CD10	MPO	CD45	CD13	CD33	CD7	CD79a	HLADR	CD117	TdT	CD20	CD22
Tegegen 2021 [33]	18	S	c	S	S	c	S	S	S	S	c	S	S			
Antillon 2016 [25]	17	S	c	S	S	c		S	S		c	S	S	c	S	S
Sengar 2009 [32]	13	S	S	S	S	S	S	S	S	S		S				S
Supriyadi 2011 [55]	13	S	c	S	S	c	S	S	S	S	c			c		S
Al-Sharifi 2016 [36]	10	S	S	S	S		S	S	S	S						
Medalla 2021 [34]	9	S	c	S	S		S				c	S		c	S	
Singh 2016 [46]	8	S	c	S	S	S					S		S			
Gupta 2016 [26]	7	S	c	S	S	c	S				c					
Navarrete 2014 [53]	5	S	c		S			S	S							
Consensus panels	**Total**	**CD19**	**CD3**	**CD34**	**CD10**	**MPO**	**CD45**	**CD13**	**CD33**	**CD7**	**CD79a**	**HLADR**	**CD117**	**TdT**	**CD20**	**CD22**
Gujral 2008 [13]	10	S		S	S		S	S	S	S		S	S			
NCCN 2022 [5]	8	S	S	S		S	S							c	S	
Yeoh 2013 [15]	6	S	c			S				S	c				S	
Mean	**10.3**															
Median	**9.5**															
Reference panels	**Total**	**CD19**	**CD3**	**CD34**	**CD10**	**MPO**	**CD45**	**CD13**	**CD33**	**CD7**	**CD79a**	**HLADR**	**CD117**	**TdT**	**CD20**	**CD22**
WHO eIVD 2019	14	S	c	S	S	c	S		S	S	c		S			
EuroFlow™ ALOT [30] (screening tube only)	8	S	c	S		c	S			S	c					

markers were ranked. In order of frequency (% of studies): CD19 (100 % of studies), cCD3 (92 % of studies), CD34 and CD10 (83 % of studies), cMPO and CD45 (67 % of studies), cCD79a and CD13 (58 % of studies), CD7 (50 %) and HLA-DR (42 %) (Table 3). While none of the 12 included publications defined this specific panel, the panel described by Gupta et al. [26] was the most similar. Gupta et al. compared HM diagnosis using their proposed minimum FCM panel with the recommended Indian panel for acute leukaemia, which included CD10, CD19, CD7, CD5, CD13, CD33, CD117, CD34, HLA-DR and CD45. [13]. The proposed panel differed from the recommended panels with inclusion of cytoplasmic CD3, MPO and CD79a. Including these markers resulted in an increase in diagnostic accuracy of 2.4 % when compared to the consensus panel up to 95.1 %, and a 20 % increase in accuracy when compared to morphology alone ($p < 0.05$). Haycocks et al. also compared a novel multi-step process including a limited FCM panel, with the initial triage performed by analysis of CD45 vs side scatter (SSC) FCM plots. [64]. Despite achieving a diagnostic accuracy of 96 %, the authors conceded that the protocol was both time consuming and complex, and that this would limit its use in RLS. Singh et al. suggested an 8 antibody panel (cCD3, cCD79a, MPO, CD19, CD34, CD10, CD117 and CD64) which gave an accuracy of 97.5 % [46]. While approaching the consensus-based panel recommended by this review, the Singh et al. study deviated by including markers recommended in lower frequency by others (CD117 and CD64). It should be noted that frequency of recommendation may not correlate well with diagnostic utility, and it is recommended that a consensus-based limited panel be verified with patient samples prior to implementation. The studies included in this review provided insufficient detail for such a meta-analysis.

4.1.2. WHO essential flow cytometry panel for leukaemia

The World Health Organisation has begun addressing resource limitations in their recent 5th edition guidelines. In addition to identifying diagnostic test recommendations that are essential, a health policy document suggesting essential in vitro diagnostic tests (WHO eIVD) was released online in 2019 [63]. Amongst many other essential tests, the policy outlined a flow cytometry panel for leukaemia comprising CD10, CD19, CD45, CD34, CD7, CD33, CD117, MPO, CD79a, and cCD3. An update in 2022 recommended the addition of HLA-DR, CD5, CD23 and CD43 to further discriminate Acute Promyelocytic Leukaemia and lymphoproliferative disorders (https://edl.who-healthtechnologies.org/recommendations/2388). This essential panel contains 14 antibodies, so is not as limited as some, and has capacity for providing a differential diagnosis for cases that are not acute leukaemia. However, it is a consensus panel that is yet to be verified in a large cohort and does not provide sufficient information for resource-stratification. There is a high degree of correlation between WHO eIVD and the limited panel constructed from this review, although CD13 is replaced with CD33 and there is additional CD117, CD5, CD23 and CD43. This panel is not stratified, so if resources are restricted it is not clear which markers should be excluded. When the 2019 essential WHO panel is considered, there is almost complete agreement with the aggregated limited panel from this review. Only 3 of the 12 limited panels reviewed were published after 2019, and none referenced the WHO eIVD basic acute leukaemia panel [5,33,34]. The only reference to this panel was found in a study by Fedoriw et al. regarding HM classification in LMIC, but was from a histopathology diagnostic perspective [65]. Individual panels showed significant deviation from the WHO basic panel, and general concordance was only seen when these panels were aggregated by this review (Table 2). Larger panels with 13–18 markers included an additional 4–8 antibodies, limiting the usefulness in RLS, while smaller panels with 5–10 markers missed between 3 and 6 antibodies from the WHO panel (Table 2). A 7 antibody panel devised by Gupta et al. was comprised entirely of 2019 recommended markers, but was missing CD33, CD7 and CD117 from a 10 marker panel [26]. Conversely, a study by Tegegen et al. included all 10 recommended markers, but also included an additional 8 antibodies and was the most comprehensive panel included in the analysis for this review [33]. While this review supports the choice of antibodies for the 2019 basic WHO eIVD panel, validation of this diagnostic panel in a large cohort of acute leukaemia patients is yet to be conducted. The 2022 updated version including CD43, CD23, CD5 and HLA-DR has 14 antibodies, which is a level of complexity that may still be unachievable by many RLS.

Table 3
Extent of concordance for defined CD markers for a limited acute leukaemia panel including studies and resource-stratified guidelines (n = 12).

Antibody	Proportion of panels including marker
CD19	100 %
cCD3	92 %
CD34	83 %
CD10	83 %
cMPO	67 %
CD45	67 %
cCD79a	58 %
CD13	58 %
CD7	50 %
HLA-DR	42 %
CD117	33 %
CD20	33 %
TdT	33 %
CD14	25 %
CD22	25 %

4.1.3. EuroFlow™ ALOT and panel verification

EuroFlow™ has published a very comprehensive FCM diagnostic algorithm, and is considered to be a reference guideline for many high-income country diagnostic services [30]. The most similar EuroFlow™ panel to the consensus-based panel defined in this review is the acute leukaemia orientation tube (ALOT). The ALOT panel is designed as a screening panel that informs more comprehensive FCM testing and is not a complete limited panel. The EuroFlow™ ALOT tube contains CD19, cCD3, CD34, cMPO, CD45, cCD79a, CD7 and sCD3 [30]. When compared to the consensus-based 10 antibody panel from this review, the only markers missing from the ALOT tube were CD10 and CD13 (Table 2). There is little utility in the inclusion of CD10 and CD13 in a screening FCM panel designed for further diagnostic stratification. This underpins the difference between a screening or orientation FCM panel and a limited FCM panel, as CD10 and CD13 would have a much greater diagnostic contribution in a limited panel for ALL and myeloid differentiation respectively. Interestingly, GBCFLUX suggested a limited-resource screening panel containing the EuroFlow™ recommended markers followed by resource-stratified disease-specific panels [14].

None of the resource-stratified recommended limited panels were validated or had published accuracy data, as has been achieved by EuroFlow™. [5,13–15]. Publication of more validation studies is clearly required to develop and verify the optimal limited panel for acute leukaemia diagnosis in RLS,

4.1.4. Screening panels as a foundation for limited flow cytometry

Screening FCM panels are devised with the intent to perform further testing once the lineage of the HM has been established. A true limited or minimal panel is developed with the intention of being the final diagnostic FCM test, to be interpreted in conjunction with morphology and cytochemistry, if available. Of the 10 guidelines included in this review, 4 provided screening panels [14,30,40,41], 3 limited panels [5,13,15], 1 consensus limited panel for CLL [37] and the remaining WHO 5th edition guidelines did not provide limited or screening panel recommendations except the online WHO eIVD [16,17]. The EuroFlow™ guidelines published in 2012 provide an acute leukaemia orientation tube (ALOT) for screening which is supported by rigorous verification with a large patient cohort [30]. Although this panel was published prior to the recommendation of limited panels by three of the four sources, the

ALOT did not appear to be used to build the limited panels for use in RLS (Table 2). Similarly, the WHO eIVD was not referenced by studies found in this review.

4.2. Lymphoproliferative disorders

Unlike acute leukaemia, there are consensus international guidelines for a limited CLL panel [37]. For completeness, this review also found 2 limited FCM panel recommendations for CLL and LPD that differed from the consensus panel with the inclusion of CD10 [5,13]. There were 7 studies that detailed CD markers for a limited CLL/LPD panel, one of which was part of a larger acute leukaemia/LPD panel [33]. The degree of agreement amongst CLL limited panels was variable, particularly the utility of CD23 vs CD22 vs CD20, and the inclusion of CD10, CD45 and FMC-7 (Fig. 2). This is an interesting finding, as less variation would be expected given the availability of a consensus limited CLL FCM panel.

4.2.1. Lymphoproliferative disorder panel vs chronic lymphocytic Leukaemia guideline panel

A consensus-based CLL/LPD panel contained 8 markers: CD19, CD5, Kappa, Lambda, CD20, CD10, CD45 and CD23 (Table 4). This panel was constructed from all studies and guidelines identified in this review as providing limited FCM for CLL/LPD/NHL in an RLS [5,11,13,33,35,37,42,44,51,53]. The National Comprehensive Cancer Network (NCCN) includes 3 separate panel recommendations for CLL, B-NHL and Burkitt lymphoma [5]. The frequency of marker recommendation depended on available resources, panel objectives and staff expertise. When the limited CLL/LPD panel from this review was compared with the CLL consensus panel by Rawstron et al., there was a slight variation [37]. CD45 was not included in the Rawstron et al. panel, and CD10 was not considered to be a 'required' marker but listed instead as 'recommended'. The addition of CD45, noted in one third of panels in this review, would allow the scatter plots to be analysed more easily, reducing operator error [64], so would be a useful addition. Similarly, CD10 would improve classification accuracy of LPDs excluding CLL, such as follicular lymphoma and diffuse large B-cell lymphoma, so would have utility in a limited general LPD panel. CD10 was included in 42 % of CLL/LPD panels in this review (Table 4), and is also included in the WHO eIVD basic panel. These facts highlight the limitations of defining a consensus-based panel, such as Rawstron et al., that is not optimised or validated with patient samples [37]. Another limitation of this panel is its focus on CLL, where there in an increasing need to differentiate other lymphoid malignancies using FCM in a cost-effective manner. There is scope to define, optimise and validate a more appropriate lymphoid FCM panel for use in RLS. The addition of CD10 and CD45 would therefore be optimal for a LPD panel not as focussed on CLL. The WHO eIVD acute panel now includes lymphoid markers CD43, CD5 and CD23 in an attempt to address this issue. As with acute leukaemia, there is a growing need in RLS to accurately diagnose not only CLL, but other LPDs. As treatment options become more widely accessible, diagnostic test accuracy must increase accordingly to keep pace [66].

Table 4
Extent of concordance for defined CD markers in a limited CLL/LPD FCM panel including studies and resource-stratified guidelines (n = 12).

Antibody	Inclusion in limited FCM panels
CD19	100 %
CD5	92 %
K/L	83 %
CD20	75 %
CD23	67 %
CD10	42 %
CD22	33 %
CD45	33 %
FMC-7	25 %

4.3. Limited flow cytometry panel accuracy

Determining the diagnostic accuracy of limited FCM panel for acute leukaemia is difficult and confounded by many factors. Often, due to resource restrictions, blood cell morphology was used as the gold-standard test and the FCM panels performed were limited. Only four studies [32,33,36,67] performed such an analysis. Ahmad et al. published a comprehensive study on diagnosis of acute leukaemia where resources were constrained, however this could not be included in the meta-analysis as limited FCM was only performed where results were considered ambiguous after morphology review [47]. Meta-analysis of the included 4 studies was confounded by the classification categories of AL. Diagnosis was therefore standardised across studies to binary classification of ALL or AML before evaluation. Sensitivity (% of patients with malignancy correctly identified) and Specificity (% of patients with no malignancy correctly identified) and the 95 % confidence intervals were calculated from the data provided in the studies. It should be noted that in these studies, FCM diagnosis of AML and ALL was considered by the authors to provide a diagnostic accuracy of 100 %, which may not be correct [27,28]. The diagnostic accuracy of morphology and/or cytochemistry when compared to limited FCM ranged from 42 % [32], to 95 % [36]. The diagnostic accuracy improvements with the introduction of limited FCM to these areas would therefore range from 5 % to 58 %. These percentages are likely to have significant error, which is unable to be calculated, due to the limitations listed above, so should be interpreted accordingly. The difference in the accuracy improvement was likely due to the choice of comparison test. Sengar et al. included an 'inconclusive' morphological classification resulting in significantly diminished accuracy. Alternately, Al-Sharfi et al. included cytochemical stains to supplement morphological classification leading to a higher comparative accuracy level and a more modest improvement by the addition of limited FCM (Fig. 3).

Gold-standard testing for many HM, where resources are not limited, is a combination of bone marrow morphology, comprehensive FCM testing and molecular analysis [16,17,30]. Ideally, to assess the improvement in diagnostic accuracy, limited FCM panels should be compared to both gold-standard testing and morphology alone. However, advanced diagnostic testing is rarely available in LMICs, even for studies specifically comparing diagnostic accuracy [19,22]. Therefore, it is difficult to draw meaningful conclusions from the 4 studies that reported data sufficient for meta-analysis. The small number of studies included in this meta-analysis and the poor quality and comparability of the studies contributed to the high variability seen in results of the analysis (Fig. 3). This analysis highlights the need for higher quality studies to assess limited panels compared to appropriate gold-standard testing.

5. Assessing current diagnostic accuracy of haematological malignancies in resource-limited settings

5.1. Haematological malignancy classification level in resource-limited settings

5.1.1. Lymphoid vs myeloid

The superseded French-American-British (FAB) morphological classification of acute leukaemia is often used in RLS to further subclassify HM categories where access to sophisticated technologies are unavailable. HM are often divided only into LPD, AML and ALL, as simple lineage determination is able to be performed using morphology and basic cytochemistry for myeloperoxidase (MPO) [22].

5.1.2. B-cell and T-cell lineage

There was considerable variation in the ability to further classify the HM to their category or class across the studies included in this review.

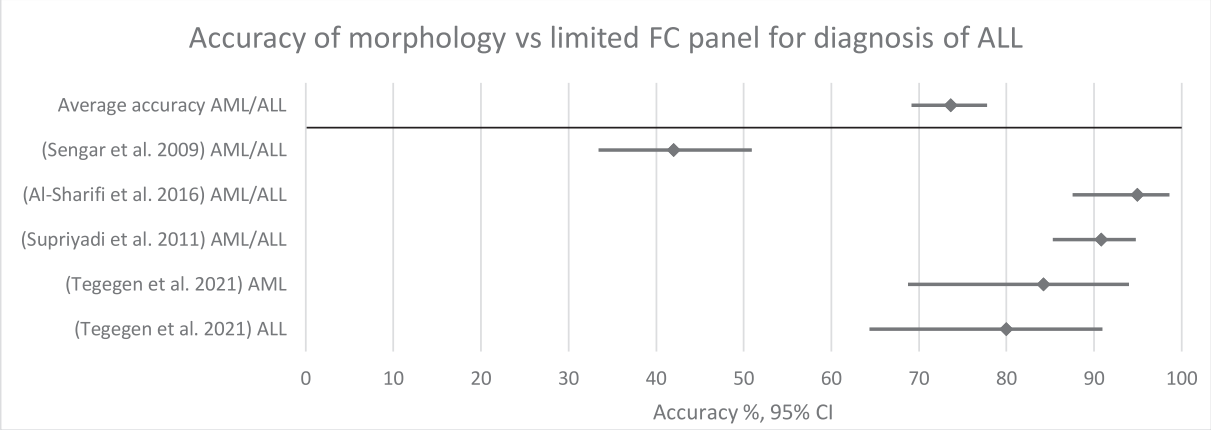

Fig. 3. Diagnostic accuracy of morphology when compared to limited panel FCM (100 % diagnostic accuracy).

All studies attempted to differentiate the maturity and lineage of the HM based on the diagnostic tests available, but further delineation to B-cell ALL, T-cell ALL or AML-FAB morphological class was only possible in a fraction of the study settings. Where there was restricted access to FCM services, 80 % of studies in this review classified acute leukaemias to lineage only. However, when there was access to FCM, 88 % of studies were able to subclassify malignant lymphocytes to B or T category, and 75 % of studies able to subclassify AML to their FAB class (Table 1). This is consistent with available literature, which agrees that use of FCM allows more specific lineage identification [15–17,30].

5.1.3. *WHO-HAEM5 hierarchy*

The fifth edition WHO classification system has introduced a hierarchical approach to help accommodate limited access to diagnostic testing. Classification order is lineage, category, family (class), type, subtype. An example of this structure is B-cell lineage, mature category, large B-cell lymphoma family, diffuse type, MYC and BCL6 rearrangement subtype [65]. As this hierarchy was only defined in 2022, it is not yet widely adopted. It is hoped that these newly defined classification terms will be standardised, allowing high quality cross-institution comparisons.

5.1.4. *Effects of disease classification level on diagnostic accuracy*

The level of achievable disease subclassification significantly affects the accuracy of a diagnostic test. As the number of disease categories increases, diagnostic accuracy decreases. In the study by Al Sharifi et al. [36], diagnostic accuracy of 92 %, when only differentiating lymphoid from myeloid, reduced to 87 % when identifying T-ALL and the FAB class of AML. Many studies in RLS that publish accuracy of acute leukaemia diagnostics use the binary options of lymphoid vs myeloid (Table 1). Oversimplification of this diagnostically inadequate system results in falsely elevated accuracy calculations, and leads to suboptimal patient outcomes when compared to the use of the minimum recommended B-cell and T-cell category level delineation of ALL [5,14]. Ability to discriminate leukaemias further than simply myeloid vs lymphoid has significant implications for treatment success [5].

5.1.5. *Disease classification level in this review*

Classifications amongst the 23 clinical studies examined for the review included AML vs ALL, inconclusive lineage, acute undifferentiated leukaemia (AUL), AML (FAB class M0-M7), B-ALL and T-ALL. Only 37 % of studies that reported FCM availability were able to discriminate ALL more specifically into B-cell or T-cell categories (Table 2). Flow cytometric analysis included an additional category of bi-phenotypic acute leukaemia (BAL), which is not diagnosable by morphology alone.

Only 4 of the 23 clinical studies provided sufficient diagnostic equivalence for meta-analysis [32,33,36,55]. These studies compared morphological diagnosis of acute leukaemia, which is readily available in many RLS, with limited panel FCM. Even within the small number of selected studies, the morphological classification level was not consistent and had to be adjusted for analysis. To compare data across studies, acute leukaemias were divided simply into AML and ALL, with BAL and AUL and inconclusive categories allocated as misidentification (Fig. 3).

5.2. *Morphology accuracy*

A study from Gupta et al. cited that discrimination of ALL from AML by an experienced morphologist is correct 70–80 % of the time [26]. Clearly, there is a range of skill with morphological diagnosis of acute leukaemia, and a detailed assessment of that variation is beyond the scope of this review, though such accuracy is likely to be significantly lower in RLS where pathologist experience and stain quality may not be equivalent to high-income countries. There are limited studies on missed diagnoses of acute leukaemia in RLS, but this is a significant issue that is difficult to detect. A unique study by Severance et al. was included in this review, and found 41 missed HM from malaria blood films in RLS [12]. This study also compared actual HM cases to those expected over 12 months in Western Kenya and estimated that 75–80 % of paediatric cancer cases remain undiagnosed. Thus, the use of limited FCM in RLS may improve the accuracy of morphology significantly. Only four studies in this review reported morphological accuracy when compared to limited panel FCM, these are the same studies compared in Fig. 2. When assessing morphology accuracy overall, the average correlation with FCM was only 74 %, consistent with Gupta et al. It is important to note that this accuracy level was achieved when classifying to the lineage level only (i.e. lymphoid vs myeloid). Diagnostic accuracy appears to decrease as the subcategories of HM available increase; therefore the accuracy of morphology alone is likely much lower than the 70–80 % determined by Gupta et al. and this review (Fig. 3). If morphology accuracy is much lower than is being reported in the literature, the improvements in diagnostic accuracy with the implementation of limited FCM will be greater than anticipated.

5.3. *Cytochemistry*

Cytochemistry has been available for nearly 100 years and is a valuable diagnostic tool for improving accuracy in HM classification. Cytochemical staining of malignant myeloid and lymphoid cells with histochemical stains such as Sudan black B, periodic acid Schiff, acid phosphatase and myeloperoxidase can assist in lineage and category determination of predominantly malignant myeloid cells [24,36,68]. This technology is often used in RLS as it is relatively inexpensive and does not require specialised instrumentation. In countries that have access to flow cytometry, however, cytochemistry has been largely

superseded [69]. Cytochemical stains are of little use when categorising lymphoid malignancies [33], but can increase accuracy considerably when used for myeloid malignancies [32]. They may also assist in determining the lineage and category of acute leukaemias where the morphology is ambiguous. In one study by Gupta et al., accuracy for acute leukaemia was increased from 75.6 % with morphology alone to 93.9 % with the addition of cytochemical staining (AML/B-ALL/T-ALL/MPAL) when compared to FCM [26]. While cytochemical stains are used routinely in many RLS, their use is often limited due to their high cost when compared to morphology alone [15,43]. When reliant on morphology without cytochemistry or FCM, it can become impossible to correctly categorise an acute leukaemia by lineage, regardless of the skill of the morphologist [25,43]. However, a morphological diagnosis should always be considered alongside additional diagnostic tests such as cytochemistry where available [24,68,70].

6. Access to flow cytometry in resource-limited settings

6.1. Defining resource-limited settings

Defining a 'resource-limited setting' is challenging. Countries may be classified as low- or middle-income by dividing the national income by the population, but wealth may be distributed unequally across many of these countries. Also, there may be non-economic factors such as life expectancy and educational attainment that contribute to resource access. A better delineation may therefore be the United Nations Human Development Index (HDI), which includes both economic and non-economic variables.

6.2. Current use of flow cytometry in resource-limited settings

One of the objectives of this review was to ascertain the barriers to accessing diagnostic services in RLS. From reviewing the literature, several themes were noted, although conclusions were tenuous due to the poor quality of the data. Firstly, only a minority of patients and clinicians in RLS had access to FCM diagnostic services. The proportion of patients with access was usually not well described and was difficult to enumerate from the publications assessed. Surprisingly, an analysis of studies quantifying the use of FCM in RLS showed that there was no significant correlation between HDI (in ascending order) and the availability of flow cytometry (Table 5). A strong correlation between HDI, a measure to quantify access to resources, and use of FCM was expected. The poor correlation between these indicators suggests that reporting of access to flow cytometry by RLS may be inaccurate due to limitations in study quality.

6.3. Resource-stratification in guidelines

A significant finding of this review is the need to stratify FCM recommendations according to resource availability. This strategy is not sufficiently implemented by the most accepted international guidelines [16,30,39]. The latest WHO 5th edition guidelines (WHO-HAEM5) were selected for inclusion in this review, as they are considered to be the foremost international consensus guidelines on the diagnosis of HM [16,17], and may still be used in resource-limited settings. While there are some accommodations in WHO-HAEM5 for FCM in areas with limited resources, documents such as the NCCN guidelines provide much more detailed resource-stratification information [5]. It should be noted that the WHO-HAEM5 guidelines and updated WHO eIVD recommendations were published in 2022, and It is not uncommon for outdated guidelines to be referenced in recent publications from RLS [23,62]. It is unlikely that the most recent International Consensus Classification (ICC) guidelines are being used in RLS [19] as they include diagnostic data from sophisticated testing, so were not included in this review [71]. Several region-specific consensus guidelines have been devised to attempt to harmonise resource-stratified, limited FCM panel recommendations where resources are limited. These are more appropriate for use in RLS [5,13,15,52].

Table 5
Access to FCM in RLS ranked by HDI - Studies reporting the use of FCM in HM diagnostics ($n = 18$).

Author	Year	Target population	Country	HDI	Access to FCM (%)
Baissa et al. [19]	2023	HM	Ethiopia	0.492	32 %
Tegegen et al. [33]	2021	HM	Ethiopia	0.492	0 %
Ogbenna et al. [20]	2021	AML	Nigeria	0.548	12 %
Korubo et al. [50]	2021	CLL	Nigeria	0.548	13 %
Okoye et al. [22]	2020	HM	Nigeria	0.548	8.3 %
Kabera et al. (91)	2013	HM	Nairobi	0.601	0 %
Shwe et al. (90)	2019	HM	Myanmar	0.608	81 %
Salaam et al. [24]	2019	ALL, AML	India	0.644	11 %
Ahmad et al. [47]	2024	ALL	India	0.644	16.1 %
Mulwu-Babu et al. [44]	2013	CLL	Kenya	0.644	0 %
Sengar et al. [32]	2009	HM	India	0.644	"Significant minority"
Varghese et al. [54]	2018	ALL <15yo	India	0.644	12 %
Hossain et al. [49]	2014	HM	Bangladesh	0.67	0 %
Sukrisman et al. [11]	2022	CLL	Indonesia	0.713	37 %–81 %
Dayton et al. [48]	2024	T-LGL	Vietnam	0.726	23.8 %
Navarrete [53]	2014	ALL <18yo	Central America	0.762	87.3 %
Mehrvar et al. [43]	2015	AML <15yo	Iran	0.78	0 %
Oh et al. (92)	2021	ALL	General RLS	LMIC	0 %

6.4. Inadequate resources for diagnosis of haematological malignancies

Guidelines that attempt to address the inequities of wealth distribution are usually stratified by resource availability. This type of diagnostic guideline provides a recommended number of FCM markers that increases with resource availability. The categories that are used to divide levels of access to resources are varied but follow a general outline. Basic resources allow provision of a minimum standard of care or adequate function of the health care system and are mandatory. A limited health service will have core resources that are recommended for major improvements in survival, and an enhanced service will have optional high-cost diagnostics and treatment options that provide lesser improvements in patient outcomes [5,14,15]. In the context of the Asian stratified guidelines, basic resources will generally not allow FCM. Limited resources allow for limited FCM, which may be defined as differentiating between T and B cells. A full FCM service with extended FCM panel would require enhanced resources. Interestingly, both the Brazilian and NCCN guidelines require FCM as a mandatory requirement even with basic resources, as FCM is essential for lineage determination of B-cell or T-cell ALL [5,14]. Therefore, of the 4 resource stratified guidelines available, the majority recommend that operating a diagnostic service for acute leukaemia without FCM is inadequate and below the minimum standard of care. In this review, 6 of the 18 studies that quantify access to FCM in RLS stated that there was no access to FCM diagnostic services, therefore providing inadequate care as defined by resource-stratified guidelines (Table 5).

6.5. Potential novel tests for resource-limited settings in absence of flow cytometry

There is a general paucity of data from studies evaluating HM diagnostics in RLS or LMIC. This was also reflected in the publication of novel diagnostic tests for HM in RLS with only 4 studies were found as part of the systematic search. Meiseles et al. conducted a study on machine learning algorithms for selecting the best treatment option for CLL patients based on a number of laboratory tests [72]. Although treatment focussed, this study was included as it explored risk stratification for patients in RLS without access to FCM and molecular tests. Machine learning may be useful in the future, but this review has confirmed that there are very few studies focussed on environments with limited resources. A study by Rego et al. explored the use of an antibody specific to acute promyelocytic leukaemia (APL), anti-PML antibody (PG-M3), using an immunofluorescence technique [73]. This technique has potential to be developed as an FCM assay. As APL is usually diagnosed using molecular testing, availability of a specific antibody could have utility in RLS that has FCM access but not molecular access. While these studies showed some promise, the resource-burden would be a likely barrier to most RLS. The availability of novel testing is limited, and significant developments would be required to approach the diagnostic utility of limited or basic FCM.

6.5.1. Immunocytochemistry

The most promising study of novel testing focussed on the use of bone marrow immunocytochemistry in comparison to FCM [74]. Flow cytometry is simply a platform, and immunophenotypes can be obtained with techniques that are more accessible. Traditional immunohistochemistry of parrafin-embedded bone marrow tissue is widely used for HM diagnosis in RLS as it is relatively simple and cost-effective [65]. Barriers to use in these areas include the lack of biopsy tissue and complexity of antigen retrival for tissue immunohistochemistry. These limitations can be mitigated if blood or bone marrow smears are used for immunophenotyping by immunocytochemistry, and the first publications of these techniques were in 1985 [75]. This study by Erber et al. noted a high degree of duplication when immunophenotyping with both immunohistochemistry and FCM. While this study advocates use of immunohistochemistry in conjunction with FCM to improve cost effective use of resources, there is potential for immunocytochemistry to be incorporated where FCM is inaccessible. A novel cell block technique has been described using immunohistochemistry to assess ALK positivity in anaplastic large cell lymphoma that may have wider applications [76]. This study describes a method for producing a peripheral blood buffy coat cell block for immunohistochemistry, which could potentially be applied to acute malignancy markers such as CD34 and MPO in settings where FCM is not available. These ideas require further exploration, as there are studies outside the scope of this review that describe immunophentype staining methods for blood films and and non-parrafinised samples that would be more logistically feasible in RLS [77–79].

6.6. Quality assurance

Quality assurance (QA) in FCM is difficult even where resources are plentiful due to a lack of conventional control samples and complex instrument settings [27]. Internal measures such as antibody validation and instrument calibration and maintenance require extensive training and education of scientists and technicians to ensure quality results. These measures may be even more important in RLS, where supply-chain delays and remote locations necessitate increased vigilance. Adherence to these practices should be monitored by external QA providers. External quality assurance is advisable for all pathology providers, but comprehensive programs are often not available in LMIC [61]. Implementation of such programs is a significant undertaking, but in the interim some success has been met with mentoring partnerships with other countries [61]. While there are difficulties in establishing and maintaining QA in an RLS, it is possible and needs to be considered as part of an FCM implementation plan.

6.6.1. Lyophilised antibody panels

Maintaining the stability of antibody panel cocktails is a common problem in FCM, and recent advances in lyophilisation techniques have begun to address this issue [80–82]. Freeze-dried antibodies offer enhanced shelf stability of up to 12 months and are often able to be transported at room temperature [80]. These are considerable advantages in RLS where supply-chain issues are common [61]. Several manufacturers offer limited panel FCM tubes, however there are drawbacks. The standard panels are structured as screening panels, often based on the EuroFlow™ recommended panels [30]. Screening panels require further comprehensive FCM for accurate disease classification. Also, standard panels require sophisticated instrumentation capable of 8, 10 or 12 colour FCM. Instruments in RLS are often 4 or 6 colour, due to the age and cost of instrumentation. While custom lyophilised panels are possible, these are significantly more expensive than conventional FCM antibodies. Improvements in efficiency and reduction of errors have been shown, but come at a cost [82]. As these products become more widely available and pricing becomes more competitive, there could be substantial benefits for RLS who can access this new technology.

7. Discussion

Diagnosis of HM is complex and requires advanced training and access to sophisticated diagnostic pathology services for maximum accuracy in all countries [16,27,30,39]. A review of the literature has found that diagnostic services are inadequate in many LMICs, and patient outcomes are inferior to high-income countries [4,19,83]. Understanding the degree of accessibility to pathology services, the barriers to access and rates of underdiagnosis and misdiagnosis is vital for improvements to be made [12,61]. However, this is difficult due to the inherent low quality of data available where resources are limited. What can be derived from reviewing the literature is identification of diagnostic approaches that are comparable across guidelines designed for RLS, and the utility of limited FCM to improve diagnostic accuracy and treatment availability. This data can be used to suggest strategies for improving the diagnostic accuracy for haematological malignancies and improving access to treatment for patients in RLS [83].

7.1. Limited flow cytometry panels reported in the literature

While the use of limited FCM panels is likely widespread, the literature fails to capture its utility and potential for optimisation. An analysis of limited FCM panels found only 15 studies examining their use, while there are almost certainly more variable panels being used in RLS. A significant limitation is the lack of comparison to gold-standard diagnostic technologies in these studies. This type of comparison is required to truly assess the improvement in diagnostic accuracy of limited FCM and indicates a significant gap in the literature that needs to be addressed.

Consensus-based panels comprising the most recommended antibody panel components were devised by this study for acute leukaemia (Table 3) and lymphoid malignancy panels (Table 4). While these provided a solid foundation for establishing a limited panel and indicated the number of antibodies that are logistically feasible in a RLS, they are not optimised or validated with patient data. While this review found that CD19, cCD3, CD34, CD10, cMPO, CD45, cCD79a, CD7 and HLA-DR are the top ranked antibodies for inclusion in a limited panel for acute leukaemia based on publications, there may be a more appropriate combination of CD markers to suit a particular austere environment. Similarly for lymphoid malignancies, a common approach in RLS is to include CD10 and CD45 in addition to traditional accepted CLL markers CD19, CD20, CD23, CD5, K/L, and CD45. As more treatment options

become available, inclusion of antibodies such as CD3, CD4 and CD8 to further discriminate other lymphomas may be justifiable. It should be noted that using only 4–6 fluorochromes, depending on the number of lasers in available instrumentation, is required to minimise technical difficulties in instrument setup and data interpretation [27,28]. Extensive comparison testing would elucidate optimal panels and provide information on stratification based on available resources. High income countries would be better positioned to perform this EuroFlow™ style validation of limited panels [30].

7.2. Provision of essential diagnostics is insufficient

Defining the diagnostic options available where resources are constrained is very difficult from reviewing the literature alone. There were very few studies outlining current practice and these also showed significant bias (Table 5). One of these studies reported a diagnostic accuracy as low as 42 % for acute leukaemia lineage classification without FCM, which would have a profound effect on treatment choice and patient outcomes [32]. Current published diagnostic practices in RLS are mostly inadequate for even basic, essential provision of healthcare services. FCM was considered the minimum requirement for adequate patient outcomes in 2 of the 3 stratified guidelines [14,15], but FCM availability was absent or severely restricted in most studies (Table 5). In addition, discrimination of B-cell or T-cell lineage category is deemed necessary to stratify patients into appropriate treatment groups, even where only basic resources are available [5,14]. FCM for lineage determination was very poorly reported by RLS, yet identification of myeloid vs lymphoid was possible only in a minority of studies, even with the availability of FCM. This may be due to inexperience, use of guidelines inappropriate to the region, or poor reporting of FCM accessibility. If access to FCM is being overreported in the literature, then this issue is even more pressing and needs further investigation.

7.3. Cost

There is no doubt that the cost of implementing FCM is a barrier for areas with limited resources. Expense is also an obstacle to some patients in higher income areas that do have access to FCM. There was only a single study in this review that examined cost-effectiveness directly, comparing the cost of comprehensive FCM to a limited panel [34]. Use of limited FCM can be less than a third of the cost of comprehensive FCM, allowing greater access and therefore improved diagnostic accuracy of HM [84]. Although other health improvement strategies may seem more cost effective, diagnosis and treatment of certain haematological malignancies in both middle-income and low-income countries has been proven to be cost effective [1]. As non-communicable diseases in RLS begin to attract the focus of the WHO, investing in LMICs would see the greatest return on investment for reducing HM burden [85]. It is projected that the increase in incidence of cancers in RLS, such as HM, may overwhelm health care systems and must be addressed [4]. Strategies to more effectively use resources that are available, such as limited FCM, will lower the barriers to improved diagnostics. This in turn may increase access to more resources by supplying more accurate health data in RLS. Both government funding and philanthropy may be required to overcome these barriers, even with optimised testing protocols.

7.4. Recommendations

7.4.1. Human resources

Comprehensive FCM requires a high degree of training and expertise in both instrument setup and data analysis. Reduction in the complexity of FCM can be achieved by using fewer antibodies in the diagnostic panels, using fewer and more stable fluorochromes, devising standard gating strategies and simplifying and expanding training opportunities. Increased collaboration between high-income countries and LMICs to devise more accessible FCM would provide substantial benefits [60,61]. A significant limitation in the data from this review was comparison of limited FCM to gold-standard comprehensive FCM. This could be overcome if more studies were conducted where resources are plentiful in collaboration with RLS. The current diagnostic research focus is to improve accuracy by expanding the complexity and range of expensive and sophisticated technologies. There is very little focus on exploring techniques that have been superseded where resources are plentiful [74]. If more research were conducted to simplify, optimise and improve the robustness of diagnostic testing for RLS, this may provide benefit to a far greater number of patients than highly specialised diagnostic research. Similarly, improved awareness of and access to FCM training programs, such as those provided by the International Society for Advancement of Cytometry (ISAC), would provide benefits to all areas that provide complex diagnostic tests, regardless of economic status.

7.4.2. Further stratification and wider utilisation of WHO guidelines

A tiered approach to diagnostics dependent on resource availability allows the most efficient use of health resources. Resource stratification was adopted by 3 of the 4 RLS guidelines for resource-restricted regions: Asia, Brazil and sub-Saharan Africa [5,14,15]. Universally accepted guidelines, such as those published by the WHO and ICC do not provide the same level of resource-stratification recommendations [16,17]. If a health service does not have access to all recommended haematological diagnostics, such as FCM, these guidelines are not appropriate to guide clinical decisions when FCM is still considered essential [86,87]. With the recent emergence of a new consensus classification incorporating more advanced diagnostic technology by the ICC, perhaps it is time to reevaluate and further increase the level of resource-stratification provided by the WHO guidelines. Inclusion of flow panel or immunophenotyping recommendations for regions with basic, core, enhanced or maximum resources would help clinicians in RLS to construct FCM panels and optimise diagnostic testing. Given the variability of availability of FCM and immunophenotyping in general within RLS, a specific working group or sub-committee within the WHO may be beneficial to produce practically useful and feasible guidelines according to the degree of disadvantage experienced by various LMICs.

7.4.3. Treatment alignment

Development of guidelines and recommendations should not only be aligned with resource availability, but also available treatment options in RLS. Emphasis on the importance of B-cell or T-cell lineage category determination in ALL or morphologically ambiguous acute leukaemia should be clear. Lineage classification of AML is far less important in RLS for the treatment options available, however diagnostics tend to focus on this deeper level of AML classification due to the wider availability of technology and expertise. An exception to this is Acute promyelocytic leukaemia, where FCM may assist diagnosis but is neither essential nor desirable for classification as defined in WHO-HAEM5 [39]. The focus needs to shift to identifying translatable immunophenotyping techniques to expand treatment options and improve patient outcomes for both acute and chronic leukaemias.

7.4.4. Novel strategies to access immunophenotyping

Novel diagnostic tests, or wide implementation of superseded immunophenotyping techniques, would enhance classification capabilities in RLS. Immunocytochemistry is easier to perform and requires no instrumentation, unlike FCM, so is a viable option for HM classification [78,88]. While bone marrow biopsies are often not performed, techniques may be performed on peripheral blood and expertise can be developed [77–79]. There are few studies exploring immunocytochemistry use in haematology in RLS, but there is great potential in revisiting this overlooked technique that has been available for 50 years. Another area for exploration is extending sample viability for referral. Fostering partnerships with regions that have access to FCM is a viable option for RLS, but there are logistical hurdles with sample storage and

refrigeration. Similarly, storage and refrigeration of reagents and antibodies can be challenging in remote areas with unreliable supply chains. New FCM technologies such as lyophilised antibody cocktails may be a solution to this problem but are still cost prohibitive for RLS. This review has found that there are many potential areas of research to improve outcomes for patients in RLS, and very little published data around HM diagnostic practices.

7.5. Limitations of the literature

While it is often concluded that more studies are required in a research area, this conclusion is exacerbated where resources are constrained. There is a substantial imbalance in research outputs between high-income countries and LMICs regarding FCM, novel diagnostic tests for haematology and validation and harmonisation of protocols. It is also noted that no publications from RLS within high-income countries were found, and more research is required in these settings. It is acknowledged that this systematic review not wholly representative of FCM availability in RLS due to the difficulties encountered in research and publication where resources are constrained.

7.5.1. Disease classification hierarchy

More studies reporting diagnostic accuracy are required, preferably using classification structures that are directly comparable, such as those outlined in WHO-HAEM5, to select the most appropriate limited FCM panels. However, due to the resource limitations of the areas involved, this is unlikely to occur. It may be more appropriate for such studies to be conducted in high-income countries to obtain the most meaningful comparative data. Ideally, gold-standard diagnostics such as comprehensive FCM, molecular and cytogenetic testing would be used to evaluate the accuracy of limited FCM panels and novel tests for HM where FCM is not achievable.

7.5.2. Standardising terms

Access to published studies and data in this area is limited by a lack of standard nomenclature and descriptive terms. Where resources are limited, region terms are inconsistent and may be described as LMIC, RLS, resource-poor, resource-constrained, austere environments, remote, regional or developing, amongst other terms. Similarly, limited panels do not have a naming convention and have been described as limited, basic, minimal, core, cost-effective, screening or essential, making them difficult to find in the literature. Resource-stratified guidelines are also referred to as resource-adapted, tiered and hierarchical. Standardisation of terms would improve access to searchable publication databases.

7.5.3. Morphology accuracy

An analysis of studies in this review also found that the challenges around morphological diagnosis of HM were not sufficiently addressed. It is well known that there is a high degree of variability in morphological accuracy which is influenced by sample quality, stain quality, morphologist skill and clinical interpretation. Therefore, it is likely that the accuracy of morphology alone is being overreported in RLS, with only one study citing that 42 % of cases were acute leukaemia that was MPO negative and had non-descript morphology [32]. If this inability to determine lineage is even vaguely representative of other RLS, then selection of appropriate treatment options would be a major challenge.

7.5.4. Lymphoproliferative disorders

Non-Hodgkin lymphomas are also disproportionately addressed in RLS. There are many publications regarding Burkitt lymphoma and CLL, and limited lymphoid FCM panels are usually designed with these entities in mind. However, with the evolving landscape regarding diagnosis and treatment of lymphoid malignancies and lymphomas, there is an emerging need to differentiate CLL from other lymphomas in peripheral blood. Inclusion of CD10 and CD45 into limited FCM panels would be an efficient strategy to address this issue but would also require additional training and education resources to be made available. These markers are included in the WHO eIVD basic panel for acute leukaemias, along with CD43, CD23 and CD5, highlighting their importance to also provide a differential diagnosis for suspected acute malignancies. Lymphomas are notoriously difficult to diagnose from peripheral blood and bone marrow morphology alone, due to the maturity of the cells, and immunophenotyping is an essential tool [16,30].

8. Conclusion and future directions

8.1. What is needed to move the field forward

Finding solutions to accessing adequate health interventions for HM in RLS is difficult. Deficiencies in access to diagnostic technologies and scientific expertise are often overlooked but are areas where substantial improvements can be made. Novel or superseded diagnostic tests which may be appropriate for RLS need to be explored and validated for use in these areas. This review has reinforced the need for a resource-stratified international consensus limited flow cytometry panels for global use now that FCM is becoming more available. Once established, these limited panels should be verified in a large cohort and compared with comprehensive FCM panels, such as EuroFlow™. In the interim, a limited consensus-based panel, informed by the literature, has been proposed by this review and may form a starting point for more targeted panel design and validation by RLS when establishing basic FCM facilities.

This review has identified high quality limited flow cytometry panels that have been recommended in only the last few years. These include the WHO essential IVD panel, the NCCN Guidelines, and the updates to WHO-Haem5 [5,16]. These guidelines are invaluable for RLS considering implementing FCM and should also be consulted and adapted for individual clinical environments and resource levels. A specific working group or sub-committee within the WHO may be beneficial to further develop resource stratification in the guidelines.

As treatments become more widely accessible, diagnostic technology must keep pace. High quality clinical studies are required to inform the judicious use of resources to provide the best outcome for patients with HM, and this may be best achieved where resources are freely available. Partnerships between resource rich and poor areas will be instrumental to simplify, validate and publish appropriate protocols and improve access to diagnostic technology globally. Accurate diagnosis can also inform enhanced outcomes in RLS by decentralisation of treatment [89].

If comparable limited FCM panels and novel diagnostics become more widely adopted, more accurate and complete health data will be derived outlining the prevalence and distribution of HM in RLS. This in turn will lead to improved health policy and contribute to the WHO and UN 2023 priority targets [83] for reducing the global burden of non-communicable diseases.

Practice points

- Flow cytometry is considered essential by WHO, NCCN, and other international expert panels for the diagnosis and treatment of many haematological malignancies. Blood and bone marrow morphology is often insufficient
- Clinicians in resource-limited settings should use validated limited flow cytometry panels where available
- Implementation of new limited flow cytometry panels may be informed by this review
- Resource-stratified guidelines are available for FCM and should be used where WHO and ICC guideline recommendations are not achievable
- Considerations should be made for participation in external quality assurance programs for FCM or formation of laboratory-based partnerships where these are not available.

Research Agenda

- Validation of limited flow cytometry panels with a large patient cohort
- Identification of emerging barriers to RLS reducing the burden of haematological malignancies
- Exploration of translating cost-effective and optimised limited flow cytometry where resources are constrained
- Viability of novel diagnostics as an adjunct to morphology alone and their effects on the improvement in diagnostic accuracy and treatment outcomes

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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