HHS Public Access

Author manuscript

Int J Lab Hematol. Author manuscript; available in PMC 2023 September 01.

Published in final edited form as:

Int J Lab Hematol. 2022 September ; 44(Suppl 1): 45–53. doi:10.1111/ijlh.13869.

Technological features of blast identification in the cerebrospinal fluid: a systematic review of flow cytometry and laboratory hematology methods

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Abstract

Involvement of the central nervous system (CNS) by acute leukemias (ALs) has important implications for risk stratification and disease outcome. The clinical laboratory plays an essential role in assessment of cerebrospinal fluid (CSF) specimens from patients with ALs at initial diagnosis, at the end of treatment, and when CNS involvement is clinically suspected. The two challenges for the laboratory are 1) to accurately provide a cell count of the CSF and 2) to successfully distinguish blasts from other cell types. These tasks are classically performed using manual techniques, which suffer from suboptimal turnaround time, imprecision, and inconsistent inter-operator performance. Technological innovations in flow cytometry and hematology analyzer technology have provided useful complements and/or alternatives to conventional manual techniques. The goals of this systematic review are to assess the current literature regarding 1) the natural history of CNS involvement by ALs and the clinical rationale for CSF testing in patients with AL, 2) the role and limitations of optical microscopy-based morphologic review of the CSF, 3) recommendations for flow cytometry as an adjunct to morphologic review of the CSF, 4) the use of hematology analyzers for CSF cell counts, and 5) CSF quality considerations in specimens from AL patients.

Introduction:

The cerebrospinal fluid (CSF) is a normally clear fluid with a cell count of <5 cells/μL,[1] consisting primarily of T-cells (~90% of cells), B-cells (~5%), and monocytes/ macrophages (~5%).[2] Optical microscopic examination, which is classically performed using a Fuchs-Rosenthal chamber for cell count followed by assessment of cell morphology using cytospin preparations, requires specialized personnel and is time-inefficient and imprecise.[1,3] Accordingly, hematology analyzers have been optimized to streamline laboratory operations by performing automated cell counts and differential counts for specimens with cell

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Competing interest: the authors have no competing interests.

counts in excess of ~30-50 cells/μL without a population of malignant cells. However, specimens with low cell counts and/or malignant cells, including leukemic blasts, are a continuing challenge to hematology analyzer technology.[4] Complementary technologies like flow cytometry may aid in the identification of abnormal cell types in the CSF. New and emerging laboratory technologies that increase the sensitivity of blast detection in the CSF may improve the ability of laboratories to identify CSF blasts, particularly in specimens with low cellularity and/or low specimen volume.

The purpose of this review is to highlight those articles in the published medical literature that address the current methods of CSF blast identification and quantitation using morphologic techniques, flow cytometry, and hematology analyzers. We also identify current challenges and future directions in this evolving field.

Materials and Methods:

Format:

We conducted this study using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist (see Supplemental Table 1).

Information sources and search strategy:

We conducted an electronic search of Medline (PubMed interface), Embase, and Web of Science using the keywords “CSF or “cerebrospinal” AND “blasts(s)”. The search was performed without date restrictions on February 3, 2022. This protocol has not been registered and individual authors were not contacted. It did not have a funding source, although investigators were supported as disclosed below.

Eligibility criteria:

We included the medical literature describing blast detection using flow cytometry and hematology analyzer technology indexed on or before February 3, 2022. There was no restriction of article type in the search; we applied restrictions of article type during the screening process to avoid inadvertently missing relevant articles.

Data extraction, screening of articles, and analysis:

Titles and abstracts of all articles identified by our searches were initially screened for relevance. Next, the full text of each potentially relevant article considered for inclusion in the analysis was reviewed for significance. The reference lists of these articles were then reviewed to identify additional articles missed by our initial searches. Additionally, a search of the citations of each of these manuscripts was performed using Google Scholar and PubMed to identify additional papers not included in the original search or indexed after the initial search date. Ultimately, a final group of articles remained for the qualitative analysis and included: 1) clinical studies and guidelines that focused on the rationale for CSF blast identification in acute leukemia 2) papers that discussed the morphologic identification of blasts in the CSF 3) articles that evaluated the role of conventional flow cytometry and minimal residual disease testing of the CSF 4) studies that evaluated the performance of hematology analyzers for blast identification 5) studies that compared morphology, flow

cytometry, analyzer, and/or molecular-based techniques in CSF blast identification and 6) studies that discussed quality-related issues in CSF blast evaluation. The content of the included studies was critically appraised by the authors, who are experts in the field of pathology/ laboratory hematology (JLF, JRB) and/or flow cytometry (JLF, JRB, CLS). Data extraction and descriptive statistics were entered/ performed using Excel (Microsoft, Redmond, WA, USA).

Results:

Study selection

Through a search of the databases, we identified a total of 4169 potential articles. Following the removal duplicate articles, we screened the titles and abstracts of the remaining articles to further identify potentially relevant articles. After assessment of all full-text articles, and the addition of articles identified by the search of references lists and citations, 108 studies met the established inclusion criteria and were included in this systematic review (see Figure 1). Due to space limitations, it was not possible to include all references in the discussion and, when necessary, the discussion was focused on articles published within the last 10 years. The complete list of studies is included in Supplemental Table 2.

Discussion:

Clinical rationale for the identification of blasts in the CSF and the natural history of CNS involvement by acute leukemia:

The rate of CNS involvement by hematologic malignancies varies between 5-15% depending on disease type and, if not effectively treated can result in permanent neurologic damage and death.[5,6] Clinical modalities that are used to assess for the presence of CNS involvement by hematologic malignancies include 1) neurologic assessment, 2) radiographic imaging, and 3) laboratory assessment of the cerebrospinal fluid (CSF), all of which are associated with variable rates of sensitivity and specificity.[5] Neurologic findings, which are the initial manifestation of CNS disease in >90% of patients, may be subtle and difficult to distinguish from symptoms due to other causes (such as chemotoxicity related to therapy).[7] Neuroimaging by MRI or other techniques, although quite sensitive, have limited specificity. Cytomorphologic examination of the CSF is considered the “gold standard” for CNS involvement by hematologic malignancy due to its high specificity, although with limited sensitivity.[5,7]

Leukemic blasts infiltrate the CNS by crossing the vascular endothelium into the arachnoid, and from there enter the CSF.[2] Once in the CSF, blasts may enter the perivascular space and the parenchyma of the brain.[2] There are three patterns of CNS involvement by acute leukemia: occult, leukemic meningitis, and myeloid sarcoma.[2] Occult involvement refers to subclinical involvement of the CSF without a mass-forming lesion. Leukemic meningitis refers to involvement of the leptomeninges by leukemic blasts with sparing of the brain parenchyma. Myeloid sarcoma refers to the presence of tumoral masses composed of blasts.[2]

The central nervous system (CNS) is the most the common site of extramedullary involvement in B-lymphoblastic leukemia/lymphoma (B-ALL), although it is overall uncommon (occurring in <5% of B-ALL patients at initial presentation and 5-10% of patients at relapse).[8] Risk factors for CNS involvement in adult acute lymphoblastic leukemia/lymphoma (ALL) include 1) age (more common in younger adults), 2) mature B-cell immunophenotype, 3) T-cell immunophenotype, 4) the translocation t(9;22), and 5) high proliferative index, high presenting WBC, and/or marked elevation of lactate dehydrogenase.[9] In children, the *BCR-ABL1* fusion (for B-ALL) and high WBC (for T-ALL) are the most significant risk factors predicting CNS disease.[10]

The effective treatment of the CNS has been a major contributor to the marked increase in survival in B-ALL patients over the past 50 years.[8] While CNS relapse was relatively common in the early days of multiagent chemotherapy, particularly in pediatric acute leukemias,[6] the rate of CNS relapse has decreased due to effective CNS prophylaxis .[11] Because of the recognized importance of CNS involvement and prophylaxis in ALL and the abundant literature that supports the clinical rationale for the significance of CSF involvement by blasts, pretreatment assessment of the CSF is a routine part of patient care.

Because CSF involvement by B-ALL is presumably more common than for other acute leukemias,[12] the majority of research studies are performed using specimens from patients with this form of acute leukemia; though it is unclear whether this data is generalizable for other leukemia types. For acute myeloid leukemia (AML), for example, the rate of CSF involvement is generally assumed to be quite low (<5% of patients).[12] However, this could represent missed diagnoses due to more subtle clinical findings, lack of consistent CSF monitoring, or other factors.[2] In contrast to pediatric ALL patients for which pretreatment CSF evaluation is routine,[13] most patients with AML do not have a pretreatment CSF evaluation; it is generally limited to patients with neurologic symptoms.[12] There is limited data to determine the significance of CSF involvement in AML. A study by Bar et al. that evaluated 327 adult patients prior to stem cell transplant showed no impact of CNS involvement on outcome.[12] The presumed low incidence and lack of significance of CNS involvement in AML has recently been challenged. Del Principe et al. collected CSF specimens from a series of AML patients at diagnosis irrespective of neurological findings, demonstrated morphologic evidence of involvement in 11%, and showed that CNS status had an independent effect on survival based on a multivariate analysis.[14] Similarly, Rozovski et al. showed decreased disease-free and overall survival in AML patients with CSF involvement.[15] These findings are at odds with other studies,[16] including a recent study by Ganzel et al. that demonstrated a much lower percentage of involvement (~1%) and failed to show a significant difference in clinical remission or overall survival between patients with or without CSF involvement at initial diagnosis.[16] After hematopoietic stem cell transplantation, which is a common therapy for suitable AML patients, the presence of blasts in the CSF does not correlate with survival but may be associated with a higher rate of relapse.[17] Taken together, it is clear that at the current time, the natural history and clinical significance of CNS involvement in AML remains an open question.[18] Risk factors for CNS involvement by AML include: 1) high levels of pretreatment serum lactate dehydrogenase, 2) high peripheral blood white blood cell count (WBC), 3) inversion of chromosome

inv (16)/ translocation t(16;16), 4) chromosome 11 abnormalities, 5) myelomonocytic/ monoblastic immunophenotype, and 6) young age.[12]

Morphologic identification of blasts in the CSF:

Morphologic evaluation of a cytocentrifuged CSF specimens was addressed in the recommendations of the 2017 College of American Pathologists (CAP)-American Society of Hematology (ASH) evidence-based guideline on the initial diagnostic work-up of acute leukemia, which was endorsed by American Society of Clinical Oncology.[19] This guideline recommends morphologic evaluation of the CSF in all ALL patients at diagnosis, at the conclusion of treatment, and when CNS relapse is suspected.[19] This guideline also recommends CSF morphologic examination of AML patients receiving intrathecal chemotherapy.[19]

Although the morphologic (cytological) identification of blasts in the CSF has been regarded by some researchers as the “gold standard” for CNS involvement in acute leukemias[5] in large part due to its high specificity (estimated at >95%),[20] its status as a gold standard point is controversial, mainly because of the limited sensitivity of morphologic review of CSF and potential problems with cell identification at this site.[8,9,21,22] Approximately 45% of acute leukemia patients with suspected CNS disease will have negative CSF morphology.[20,23] Morphologic review of CSF specimens is classically performed using a Fuchs-Rosenthal chamber with microscopic review for determining cell count and differential, respectively.[4] Because of the normally low cell count of the CSF,[1] morphologic assessment of this site is time-consuming and laborious. Since morphologic review is often performed on cytospin preparations, distortion of cell morphology may make the distinction of blasts from other cell types such as reactive lymphocytes and germinal matrix cells challenging (Figure 2).[24–26] Factors such as time-in-transit, specimen preparation, and cytocentrifuge instrumentation/ rotor speed may impact the quality of cell preparations, and efforts to harmonize these systems may result in a more reliable product.[27] The use of albumin or other proteins to stabilize cell membranes has been recommended to improve cell preservation.[27] Despite efforts such as a scoring system to more precisely categorize difficult cases,[28] sensitivity and imprecision remain a problem.

Nevertheless, morphologic assessment of the CSF is a mainstay in the clinical staging of acute leukemias, and cellularity of the specimen, presence of contaminating red blood cells (i.e. from a traumatic tap), and identification of blasts are included in this system. Based on these variables, CSF specimens are assigned to one of the following categories: (see Table 1).[8] Although this classification system was originally established for pediatric ALL clinical trials, it is now widely employed irrespective of patient population and leukemia type. The widely accepted definition of CNS involvement by acute leukemia is that of CNS3, i.e., WBC of ≥5/μL with morphologic identification of blasts.[11] The significance of CNS2 is controversial and may be impacted by disease type, treatment protocol, and other factors.[11,29] Some studies of pediatric B-ALL have shown similar prognosis for patients with CNS1 and CNS2.[30]

Blast identification by flow cytometry of the CSF:

With recognition of the limitations of morphologic evaluation of CSF blasts, an obvious potential ancillary technique to aid in blast identification at this site is flow cytometry. The CAP-ASH guideline recommends the use of multiparameter flow cytometry analysis for CSF specimens from patients with acute leukemia, particularly in situations where morphologic findings are ambiguous.[19] Flow cytometric analysis of the CSF has been noted to increase the sensitivity of blast identification in many studies[23] and can identify abnormal cell populations in the CSF even in specimens with very low cell counts (e.g. total WBC hemocytometer counts <10,000); current techniques are able to detect 1 abnormal cell from 10,000 total WBC count.[31,32] Accordingly, some authors maintain that flow cytometry has subverted morphology as the "gold standard" for CSF involvement by a variety of pathologic conditions.[33] As an example of this increase in sensitivity, Cancela et al. noted an increase in CSF involvement from 4% to 17% for specimens tested by flow cytometry in addition to morphologic analysis in their study of 72 specimens from pediatric B-ALL patients.[8] The increased sensitivity of flow cytometry versus cytomorphology for detection of blasts has been reported by others[23,34–42] and appears to have clinical significance. For adult B-ALL patients without morphologic evidence of disease but having a blast population identified by flow cytometry, the abnormal flow cytometry result was associated with an adverse outcome.[9,20,43] Pediatric patients with flow cytometric evidence of continued CNS disease appear to also be at increased risk of relapse.[44] Together, these studies indicate that flow cytometry may serve as a useful adjunct to morphologic review, particularly in situations where a distinct abnormal immunophenotype is identified or where morphologic findings are ambiguous.[8,21,45] In specimens where atypical mononuclear cells are difficult to distinguish as "atypical" lymphocytes or blasts, immunophenotypic identification of cells by flow cytometric analysis of the CSF may help to successfully categorize the cells.

Although the increased rate of detection of blasts in the CSF using flow cytometry is now recognized, the clinical significance of this finding needs established. To this end, two recent studies from the Nordic Society of Pediatric Hematology and Oncology have found that flow cytometry is useful in distinguishing CNS1 pediatric ALL patients at risk of relapse; with patients with CSF involvement as determined by flow cytometry had elevated risk of disease recurrence.[42,43]

Over the past several years, developments in flow cytometry have resulted in next-generation flow cytometry platforms that allow 8 or more color combinations per tube, as exemplified by the work of the EuroFlow consortium and by commercially available flow cytometry systems such as ClearLLab (Beckman Coulter, Indianapolis, IN USA).[46,47] The possibility of testing individual cell populations with ≥8 antibodies can maximize the diagnostic yield of low-cellularity specimens such as the CSF and likely will further enhance the usefulness of flow cytometric analysis of these specimens. However, these assays employ multiple tubes, and CSF cellularity can be quite low and insufficient for splitting samples multiple times for next-generation flow cytometry.

Minimal/measurable residual disease (MRD) testing by flow cytometry has an established role in B-ALL, AML, and other hematopoietic malignancies due to its ability to detect small levels of disease involvement, and it has an emerging role in blast identification in CSF

specimens. One potential use of MRD flow cytometry is the early identification of isolated CNS relapse (iCNSr) in B-ALL. Since most pediatric B-ALL protocols require a threshold CSF WBC >5 $\times 10^6$/L (or >5 cells/μL) in addition to morphologic identification of blasts on a cytospin slide, patients may need to wait on average 16 weeks before this cell count is reached, according to a recent study.[48] Agrawal et al. tested 4 pediatric B-ALL patients with consistently abnormal post-treatment CSF cytomorphology specimens using a B-ALL MRD flow cytometry approach: all were abnormal and had the immunophenotype of the patients' bone marrow blasts, thus establishing iCNSr several weeks early.[48]

One innovative strategy in flow cytometry is the use of dried antibodies, which allow for easier storage and minimal pipetting. Bouriche et al. evaluated the use of an 8-color dried antibody MRD assay and demonstrated good agreement between the conventional and dried antibody assays in 2 CSF specimens that they tested.[49] Other improvements in technologies related to flow cytometry have the potential to enhance the role of these approaches in the diagnosis of AL in the CSF. One of these technologies in Chipcytometry, which uses slide-based automated epifluorescence microscopy of cells immobilized on a solid surface (the "chip") with multiplexing technology for protein detection and has been used on fluids such as peripheral blood and CSF.[33,50] An advantage of Chipcytometry is that cells preserved on chips can be stored for as long as 20 months and can be later tested with different antibodies.[33] A recent study by Hummert et al. showed good agreement with flow cytometry when testing CSF specimens.[33] Spectral flow cytometry is another promising technique that may improve the yield of low cellularity specimens such as the CSF.[47] In spectral flow cytometry, all emitted light is detected. Resolution of different fluorochrome-conjugated antibodies is successful because the pattern of light that is emitted (the spectral pattern) is unique to each fluorochrome. This technology makes multiparameter analysis of >30 colors per tube possible, thus vastly increasing the amount of data that can be acquired from a low cellularity specimen such as the CSF.[47] Disadvantages of these new technologies include: 1) the need for specialized equipment beyond a standard flow cytometer, 2) possible regulatory issues for technologies not yet cleared by the Food and Drug Administration (USA), and 3) need for staffing by technologists with specialized expertise.

The role of hematology analyzers in blast identification of CSF specimens:

Improvements in hematology analyzer technology and image analysis have greatly facilitated the process of CSF analysis in clinical laboratories, and the current state of the art has been the subject of a recent review.[4] A major limiting factor of automated hematology devices is their limited performance in low cell count fluids such as the CSF. Currently, the lower limit of reliability of the WBC is ~20 cells/μL.[4] Therefore, it is generally recommended that cell counts below the range of ~30 cells/μL should be verified by microscopic review.[4] Moreover, due to technological limitations regarding cell identification that particularly affects the CSF in contrast to more cellular body fluids,[51] automated unsupervised counting of blasts in CSF is not yet practical. For these reasons, hematology analyzer technology is primarily employed as a screening procedure, albeit an important one.[4]

A number of studies have compared the functionality of various hematology analyzers operating in body fluid mode and have compared the results of cell count and cell identification to morphologic examination (Table 2).[51–53] All demonstrated a good correlation of WBC between the analyzer and manual counts, although it should be noted that correlation for the Sysmex XN deteriorated in the range of WBC <5/μL. Buoro et al.[54] demonstrated excellent agreement between the Sysmex XN and microscopy (AUC=0.98) for WBC. Jaime-Perez et al. compared the performance of the Sysmex XT-4000i to optical microscopy either by conventional microscopy or evaluation of cytospins in a small cohort of ALL CSF specimens[36] and showed that the automated approach had a comparable sensitivity and specificity to the traditional manual approach and a much greater sensitivity, but lower specificity than cytospin review.[36] There appears to be limited information in the literature regarding the performance of digital morphology analyzers in CSF fluids; a study by Riedl et al.[55] showed a good correlation between the CellaVision (Cellavision, Lund, Sweden) system and manual microscopy for pre-classification accuracy (90%) and the post-classification correlation coefficient (0.92-0.99).

In view of the limited capacity for the current generation of hematology analyzers to reliably produce an accurate cell count for very low cellularity specimens, specialized CSF cell counting devices have been introduced to the market. An example of this is the GloCyte system (Advanced Instruments, Norwood, MA, USA).[56,57] The GloCyte system concentrates the CSF specimen and stains RBCs with fluorochrome-labeled antibodies and total nucleated cells (TNCs) with nucleic acid dyes.[57] Sandhaus et al. demonstrated that the GloCyte system had a comparable performance to the Sysmex XE-5000 on clear CSF specimens and improved imprecision compared to the manual cell counting technique.[56] Hod et al. showed that the GloCyte system can detect as few as 1 total nucleated cell (TNC) per μL in the CSF, and that reliable cell counts can be performed as low as 3 TNCs/μL.[57] Although morphologic review is necessary to classify cell types, the use of specialized CSF cell counters such as the GloCyte system may provide for more reliable cell counts in low cellularity CSF specimens and therefore may permit laboratories to more consistently distinguish specimens on each side of the 5 blasts/ μL threshold. The identification of RBCs by fluorescence-labeled antibodies may allow for improved performance in the identification of RBCs for the classification of specimens into the **TLP pos** category.

In summary, hematology analyzers appear to have a performance comparable to conventional Fuchs-Rosenthal derived cell counts in CSF specimens, at least for specimens with reasonable cellularity. For laboratories with high volumes of CSF specimens, hematology analyzers and specialized CSF counting systems may provide improved efficiency without unnecessarily compromising quality. Further, systems that include digital morphology may be useful for CSF specimens.

Quality considerations:

We have recently reviewed quality issues in flow cytometry, including considerations for the CSF.[47] Based on a study by Glantz et al. optimal CSF specimens should have the following characteristics: 1) Volume of ~10.5 mL (to maximize morphologic review), 2) minimal processing delays, and 3) repeat analysis in patients with negative test results and clinical

suspicion of involvement.[58] Due to practical considerations, particularly in children, it may be difficult to obtain a specimen with a volume of 10.5mL.[23]

For CSF specimens, the most obvious preanalytical factor with diagnostic implications is hemodilution.[59] This problem is recognized in the classification of CSF specimens, with specimens categorized as TLP pos or TLP neg when they have ≥10 RBCs/μL; for such specimens, it is unclear whether 1) in the case of TLP pos specimens, if the blasts originated from the blood or CSF, 2) in the case of TLP neg specimens, whether the CSF was adequately sampled or was excessively diluted by peripheral blood. There are several potential reasons for hemodilution of specimens. These include 1) tube order, 2) technique-related issues with lumbar puncture (LP) procedure technique, 3) needle diameter, and 4) patient-related comorbidities such as obesity.[47,60] Apart from its diagnostic implications, there is a potential, albeit controversial, link between traumatic lumbar puncture (TLP) and outcome that suggests frequent surveillance CSF specimen collection could be hazardous.[61–63] Based on a multivariate analysis of 546 pediatric ALL patients, Gajjar et al. reported that patients with ≥2 sequential TLPs had poor 5-year survival;[61] the authors hypothesized that leukemic blasts from the blood may have seeded the CSF.[61] Subsequent studies have indicated this risk may be ameliorated with intrathecal chemotherapy.[64] Ultimately, these findings are controversial and have not been replicated by others.[30,65]

Timely testing of CSF specimens is also an important quality factor. Significant loss of cells from CSF specimens may occur as soon as 30 minutes after LP, which can have significant implications for morphologic evaluation of cells and analysis of rare populations by flow cytometry.[66] A variety of cell fixation and preservation reagents currently on the market appear to have a variable performance for CSF specimens.[67] The addition of media such as Roswell Park Medical Institute (RPMI) 1640, or TransFix® (Cytomark, Buckingham, UK) can help preserve cells for flow cytometry.[66,67] Regardless of cell preparation method, it is recommended that cell count and morphologic assessment of CSFs be performed within 1 hour of collection to avoid significant cell loss.[68]

Limitations:

Despite efforts to make our search as comprehensive as possible, it may not have included very recent publications not yet listed in databases. This search methodology may have also missed articles that did not list CSF and/or blasts in title and/or abstract. Despite the manual search of references and a comprehensive manual search of citations of each included paper, we may have missed clinical studies in which CSF technologies were mentioned but were not a major focus.

Conclusions:

Based on our review of the extant literature on the topic of blast identification in the CSF, it is clear the clinical laboratory tests of CSF involvement play a central role in risk stratification and clinical management of patients with acute leukemias. The evidence for this claim is most clear in pediatric B-ALL; studies focused on other patient populations, including adults and patients with AML are less prevalent in the literature. In addition,

improvements in instrument technology have the potential to provide better performance for the classification of CSF specimens. Current challenges include: 1) more precisely characterizing the natural history of AL involvement of the CNS, particularly in AML, 2) improvements in automated cell count technology to provide precise cell counts in low cellularity specimens, 3) more concretely defining the role of flow MRD testing of CSF specimens to potentially improve disease detection and delineate treatment success or failure, and 4) improved recognition of the importance of specimen quality by clinicians and laboratory personnel to maximize specimen yield and improve healthcare delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

JRB is supported by the National Institutes of Health (NIH) Office of the Director (DP5 OD028125), NIH National Institute of Allergy and Infectious Diseases (NIAID, R01 AI168044), and Burroughs Wellcome Fund Career Award for Medical Scientists (#1019648), is a consultant for DeciBio and Flagship Pioneering, has received royalties from Springer Science+Business Media, and has pending patent applications related to clinical diagnostic testing.

Funding statement:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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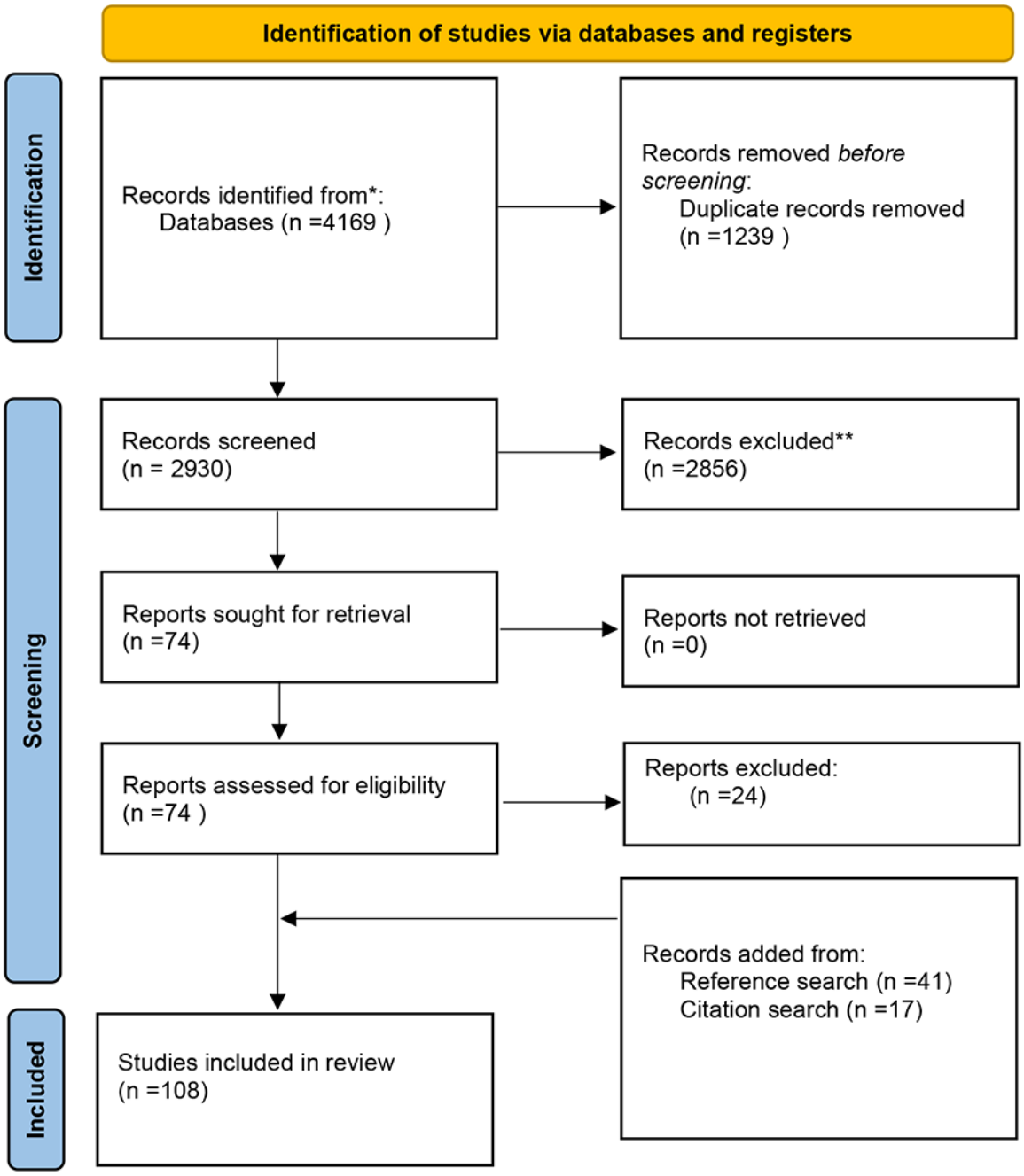

Figure 1:
PRISMA 2020 flow diagram showing the search strategy used in this review.

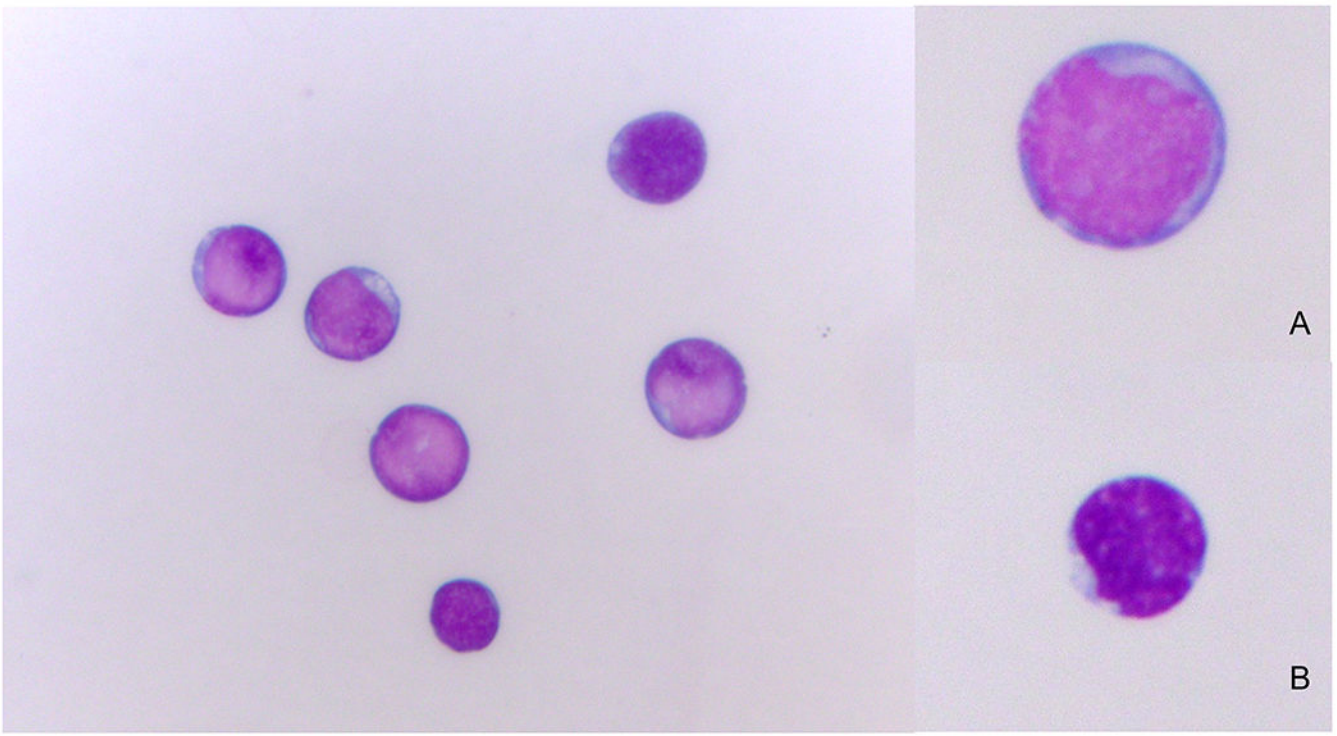

Figure 2:
Images from a CSF cytocentrifuge specimen from a patient with acute myeloid leukemia (Wright-Giemsa, original magnification x1000). Insert A shows a blast, Insert B shows a lymphocyte.

Table 1:

Classification of cerebrospinal fluid involvement by leukemic blasts (from Cancela CSP, Murao M, Assumpcao JG, et al. Immunophenotyping of the cerebrospinal fluid as a prognostic factor at diagnosis of acute lymphoblastic leukemia in children and adolescents. *Pediatr Hematol Oncol.* 2017;34(2):53-65).

Term	Definition
CNS1	atraumatic lumbar puncture, absence of blasts
CNS2	atraumatic lumbar puncture, leukocyte count <5/μL, presence of blasts
CNS3	atraumatic lumbar puncture, leukocyte count ≥5/μL, presence of blasts
TLP neg	negative traumatic lumbar puncture (≥10 red blood cells/μL), absence of blasts
TLP pos	positive traumatic lumbar puncture (≥10 red blood cells/μL), presence of blasts

Table 2:

Hematology analyzer studies reporting cell counts on cerebrospinal fluid: 2014 to present.

Name	Year	Analyzer	Number of specimens	Correlation with manual WBC, (R^2 or Cohen's kappa)
Cho[50]	2014	Sysmex XN	77	0.88 (R^2)
Seo[52]	2015	Sysmex XN	47	0.88 (R^2), Poor correlation for WBC when <5/μL, (R^2=0.56)
Buoro[51]	2019	Sysmex XN	132	0.83 (kappa)
Buoro[51]	2019	Mindray BC-6800	132	0.76 (kappa)