

Annals of Leukemia Research

Review Article

Measurable Residual Disease Monitoring by Multiparameter Flow Cytometry and Relapse Prediction in Adult Acute Myeloid Leukemia

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Received: 15 February 2024; Accepted: 03 May 2024; Published: 12 August 2024

Citation of this article: Farkas, Z., Roth, U., Fitzgerald, C. (2024) Measurable Residual Disease Monitoring by Multiparameter Flow Cytometry and Relapse Prediction in Adult Acute Myeloid Leukemia. *Ann Leuk Res*, 5(1): 04-06. doi: 10.5281/zenodo.22227540

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Summary

Roughly half the adults who achieve complete remission from acute myeloid leukaemia go on to relapse, and morphological remission cannot distinguish them in advance. Flow cytometric residual disease can, but the threshold that defines a positive result was set by consensus rather than derived from data, and the largest analysis to test it found it wanting.

Objectives: To review the published evidence on measurable residual disease assessed by multiparameter flow cytometry in adult acute myeloid leukaemia: what it predicts, when it should be measured, how the 0.1 per cent threshold performs, and what a positive or negative result should change.

Material and Methods: Narrative review of cooperative group trials, consensus documents, methodological analyses and surveillance cohorts indexed in PubMed and the major haematology journals. Studies were eligible if they reported the prognostic value of flow cytometric residual disease in a defined adult acute myeloid leukaemia population, compared timepoints or thresholds, or examined assay performance. No new patient data were generated and no patients were recruited; every estimate quoted is one published by the original investigators.

Results: The European LeukemiaNet defines positivity at 0.1 per cent of CD45-expressing cells carrying the target immunophenotype. Applied to 2,051 patients across four cooperative group cohorts in which residual disease did not guide treatment, dichotomising at that value produced suboptimal relapse-risk stratification, and levels below it remained prognostically informative. In a surveillance cohort of 136 samples preceding relapse by a median of 2.45 months and 155 taken during sustained remission, a threshold of 0.040 per cent gave sensitivity of 74 per cent and specificity of 87 per cent, against 51 and 98 per cent at 0.1 per cent. Assessment after two consolidation cycles outperformed assessment after induction, with hazard ratios of 3.635 for relapse-free and 3.511 for overall survival. Among 273 patients assessed at both cycles, 91.8 per cent of those negative after cycle 1 remained negative, while only 53.2 per cent of those positive remained positive.

Conclusion: A negative result is stable and reliable; a positive result at one timepoint is frequently not confirmed at the next. The 0.1 per cent threshold buys specificity at a cost of missing roughly half of impending relapses, which is defensible only if the intended action carries substantial risk.

Abbreviations: AML – Acute Myeloid Leukaemia, MRD – Measurable Residual Disease, MFC – Multiparameter Flow Cytometry, LAIP – Leukaemia-Associated Immunophenotype, DfN – Different-from-Normal, ELN – European LeukemiaNet, CR – Complete Remission, RFS – Relapse-Free Survival, OS – Overall Survival, alloHCT – Allogeneic Haematopoietic Cell Transplantation, LSC – Leukaemic Stem Cell, WBC – White Blood Cells.

Keywords: Acute Myeloid Leukaemia; Measurable Residual Disease; Flow Cytometry; Relapse Prediction; European LeukemiaNet

Introduction

About half of adults with acute myeloid leukaemia who achieve complete remission ultimately relapse, and the leukaemia responsible survives treatment at levels below what morphology can detect [1]. Residual disease measurement exists to find it, and flow cytometry is the method applicable to the largest share of patients because it does not require a patient-specific molecular marker [2,3].

The approach identifies cells carrying a leukaemia-associated immunophenotype, or alternatively cells deviating from normal maturation patterns, and expresses them as a proportion of leucocytes [2,4]. Its prognostic value was established across cooperative groups more than a

decade ago, in older patients [5] and in trial populations of all ages [1,6], and it is now embedded in international recommendations [7].

What has proved harder is defining what counts as positive. The European LeukemiaNet Working Party settled on 0.1 per cent of CD45-expressing cells with the target phenotype, applied uniformly regardless of the assay used [2,4]. That figure was a consensus position rather than an empirically derived optimum, and it has since been tested directly [8].

This review sets out what flow cytometric residual disease predicts, when it is best measured, how the threshold performs against the data, and what follows for the decisions it is meant to inform.

Material and Methods

Search strategy: PubMed and the archives of the major haematology journals were searched for studies of flow cytometric residual disease in adult acute myeloid leukaemia. Search terms combined acute myeloid leukaemia with measurable residual disease, minimal residual disease, multiparameter flow cytometry, leukaemia-associated immunophenotype, different-from-normal, threshold and relapse prediction. Reference lists of retrieved consensus documents and cohort analyses were screened by hand.

Eligibility: Studies were included if they reported the prognostic value of flow cytometric residual disease in a defined adult population, compared assessment timepoints or positivity thresholds, or examined technical determinants of assay performance. Molecular residual disease studies are cited for comparison and identified as such. Paediatric cohorts are excluded except where they established the method.

Data extraction and handling: Design, cohort size, timepoint, threshold applied, analytical approach and reported effect estimates were extracted as published. Timepoint and threshold are stated alongside every figure, because a residual disease result carries no interpretation without both. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 4 and Tables 1 to 3 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

The prognostic signal is well established: Flow cytometric residual disease measured after each course of therapy separated patients with favourable outcome from those with high relapse rates and inferior relapse-free and overall survival in a cooperative group study, and remained an independent prognostic factor in the whole population and within the intermediate-risk cytogenetic subgroup [1]. Multivariate analysis after cycle 2, the point at which consolidation decisions are made, confirmed that values above 0.1 per cent predicted relapse after adjustment for consolidation treatment and

time to remission [1].

Parallel work established the same relationship in older patients treated with less intensive regimens [5], and subsequent analyses confirmed that residual disease adds information beyond clinical response [6] and beyond cytogenetic and molecular classification at diagnosis [9]. Early assessment during aplasia has also been shown to carry prognostic weight [10], as has early blast clearance measured morphologically [11].

Timing: later is stronger: The most direct comparison of timepoints assessed 492 patients after induction and 421 after two consolidation cycles. The later timepoint proved the superior indicator, with independent prognostic significance for relapse-free survival (HR 3.635, 95% CI 2.433-5.431, $p < 0.001$) and overall survival (HR 3.511, 95% CI 2.191-5.626, $p < 0.001$) (Figure 2) [12]. Combining flow cytometric with molecular measurement refined stratification further, and adding fusion transcript monitoring produced a measurable reclassification improvement [12,13].

That finding sits awkwardly against clinical need. Consolidation and donor search decisions are taken after induction or after cycle 2, not after consolidation is complete, so the timepoint with the strongest prognostic signal arrives after the decision it would inform. Recommendations accordingly specify assessment after induction while acknowledging the debate about optimal timing [2,4].

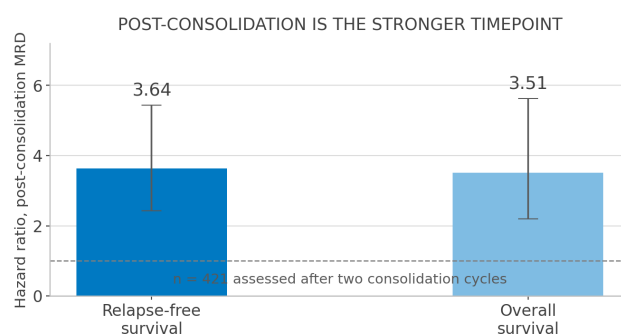


Figure 2: Hazard ratios for post-consolidation residual disease in 421 patients [12]

A negative result is stable; a positive one often is not: Among 273 patients assessed after both cycle 1 and cycle 2 within four cooperative group trials, 196 (71.8 per cent) were negative after cycle 1, and 180 of those (91.8 per cent) remained negative after cycle 2. Of the 77 positive after cycle 1, only 41 (53.2 per cent) were still positive after cycle 2 (Figure 3) [14].

The asymmetry matters practically. A negative result after one cycle is likely to persist and can reasonably inform planning, which the same analysis exploited by modelling a substantial cost reduction from initiating donor search on the cycle 1 result rather than for every patient [14]. A positive result after one cycle is closer to a coin flip as to whether it will still be positive after the next.

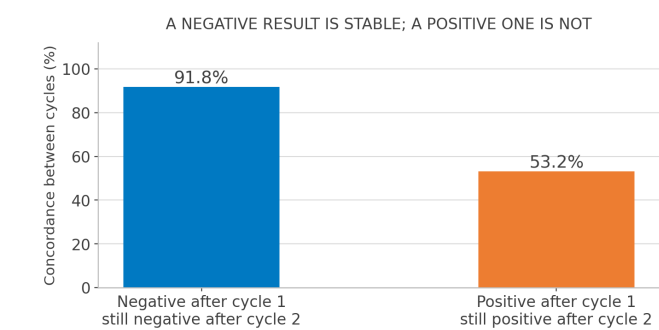


Figure 3: Concordance of residual disease status between cycle 1 and cycle 2 in 273 patients [14]

Study	n	Focus	Principal reported finding
Terwijn et al. 2013 [1]	—	Prognosis	Independent prognostic factor after each course
Freeman et al. 2013 [5]	—	Older patients	Treatment response by flow predicts outcome
Chen et al. 2015 [6]	—	Clinical response	80% relapse at one year with MRD-positive remission
Schuurhuis et al. 2018 [2]	Consensus	ELN	0.1% threshold defined
Liu et al. 2021 [12]	492 / 421	Timepoints	Post-consolidation superior to post-induction
Rodríguez-Arbolí 2024 [8]	2,051	Threshold	0.1% dichotomy suboptimal across four cohorts

Table 1: Principal studies reviewed, with focus and the finding as reported by the original investigators

The threshold does not survive testing: The 0.1 per cent cutoff was adopted assay-agnostically, meaning it applies regardless of how the measurement was made [2,4]. Four well-annotated cohorts from British, Italian, Dutch and North American groups, comprising 2,051 patients in whom residual disease results did not guide treatment, were analysed to test it. Dichotomising at 0.1 per cent produced suboptimal relapse-risk stratification, and values below that level remained prognostically informative, suggesting lower assay-specific thresholds would perform better [8].

Surveillance data quantify the trade-off. In a cohort of 136 marrow samples preceding relapse by a median of 2.45 months and 155 taken during sustained remission, a threshold of 0.040 per cent gave clinical sensitivity of 74 per cent and specificity of 87 per cent for relapse, against 51 per cent and 98 per cent at the 0.1 per cent threshold (Figure 1) [15]. Moving the cutoff therefore converts roughly a quarter of missed relapses into false alarms.

A related proposal defines the low-level cutoff by the assay's limit of quantification rather than by a fixed percentage, on the basis that around 30 per cent of patients called negative at 0.1 per cent nonetheless relapse [16]. A threshold of 50 phenotype-positive events among at least 500,000 leucocytes has been suggested on that reasoning [16].

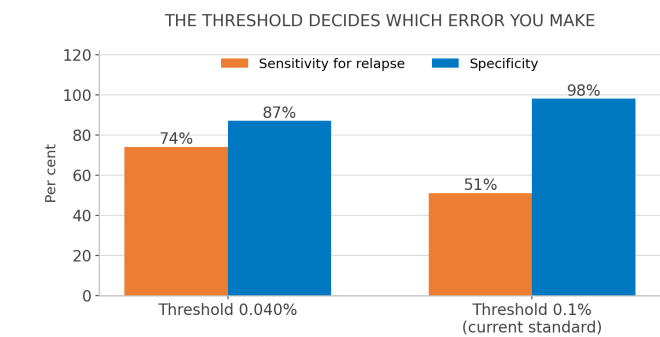


Figure 1: Sensitivity and specificity for relapse at two candidate thresholds in a surveillance cohort [15]

What a positive result implies without transplantation: The clinical weight of a positive result is best illustrated by outcome without intervention. Patients not receiving allogeneic transplantation had an 80 per cent probability of relapse within one year if complete remission was accompanied by detectable residual disease, against 20 per cent if it was not (Figure 4) [6].

Transplantation improves on that but does not abolish it. Pre-transplant residual disease predicts inferior post-transplant outcome, particularly relapse-free survival [17], and conditioning intensity interacts with peritransplant residual disease dynamics [9]. Achieving a residual disease-negative remission before myeloablative transplantation predicts better outcome than achieving remission alone [18].

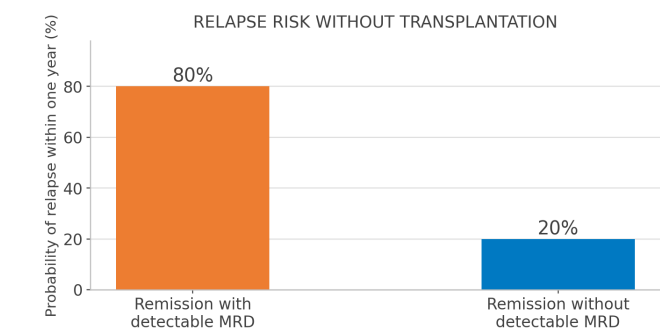


Figure 4: One-year relapse probability by residual disease status in patients not proceeding to transplantation [6]

Threshold	Sensitivity	Specificity	Consequence
0.1% (ELN standard)	51%	98%	Misses about half of impending relapses [15]
0.040%	74%	87%	Detects more, at the cost of false alarms [15]
0.036% (computational)	Equivalent	—	Unsupervised analysis matches manual gating [15]
Limit of quantification	—	—	Assay-specific rather than fixed [16]

Table 2: Candidate thresholds and what each delivers in a surveillance setting

Kinetics and what surveillance can detect: Relapse is not instantaneous, and the rate at which residual disease expands determines how much warning surveillance provides. Median relapse kinetics were 0.78 log₁₀ per month overall and significantly faster at 0.92 in FLT3-mutated disease [15]. Marrows preceding relapse were sampled a median of 2.45 months beforehand, which defines the practical window for pre-emptive intervention [15].

Computational analysis of the same samples generated optimal thresholds of 0.036 and 0.082 per cent with clinical sensitivity equivalent to manual gating, and aberrancies within HLA-DR-low or CD34-positive CD38-low stem-cell-like subpopulations contributed the greatest accuracy at 56 per cent sensitivity and 90 per cent specificity [15]. Leukaemic stem cell frequency measured at diagnosis independently predicts outcome [19], which makes the convergence of these two lines of work notable.

Where molecular measurement fits alongside: Flow cytometry is not the only route to residual disease, and the comparison shapes when it should be used. Sequencing-based detection of persistent mutations after induction predicts relapse and reduced survival independently of flow cytometric findings [24], and quantitative monitoring of NPM1 transcripts in standard-risk disease has been shown to carry strong prognostic weight [25]. Combining flow with molecular measurement refines stratification beyond either alone [12].

The practical division is one of applicability. A molecular marker gives greater sensitivity but exists in only a subset of patients, while flow cytometry applies to nearly all [2,3]. Where both are available the molecular assay is generally the more sensitive; where only flow is available, the questions of threshold and timing examined above become the whole of the problem.

Interpretation is complicated by clonal evolution in both compartments. NPM1-mutated disease can relapse with wild-type NPM1, driven by persistent clonal haematopoiesis that a marker-specific assay would not follow [22], and clonal evolution under FLT3-directed therapy has been documented directly [23]. A residual disease assay tracks the clone it was designed against, and relapse does not always arise from that clone.

Method	Applicable to	Practical characteristics
Multiparameter flow cytometry	Nearly all patients	Operator-dependent; threshold contested; result in hours [2,8,20]
Quantitative PCR of fusion or NPM1 transcripts	Defined molecular subsets	More sensitive; requires a trackable marker [12,25]
Next-generation sequencing	Most patients	Detects persistent mutations; clonal haematopoiesis confounds [22,24]
Leukaemic stem cell phenotyping	Research settings	Independently prognostic; contributes most accuracy in surveillance [15,19]

Table 3: Methods for residual disease assessment and the practical characteristics of each

Whether the result should change treatment: Prognostic value and clinical utility are separate claims, and the second is less well established than the first. Reviews addressing whether residual disease already guides therapy have concluded that it informs risk assessment more confidently than it directs any specific intervention [21]. The strongest case is for transplant allocation, where pre-transplant status predicts post-transplant outcome and a negative result before conditioning is associated with better survival [17,18].

Even there the direction of action is debatable. A patient with detectable disease before transplant has a worse outcome after it, but the alternative is not a better outcome without it, so the result may argue for modifying conditioning or for additional pre-transplant therapy rather than against proceeding [9,17]. That is a different use of a prognostic marker from the one usually implied.

Discussion

Two findings should change how a result is read. The first is that the threshold defining positivity was set by consensus and does not survive empirical testing: across 2,051 patients in whom the result did not influence treatment, dichotomising at 0.1 per cent stratified relapse risk suboptimally, and levels beneath it still carried information [8]. The second is that a positive result is far less reliable than a negative one, with only 53.2 per cent of cycle 1 positives still positive after cycle 2 against 91.8 per cent persistence among negatives [14].

Taken together these argue for reading the negative result confidently and the positive one provisionally. A patient negative after cycle 1 is very likely still negative after cycle 2 and planning can proceed on that basis; a patient positive after cycle 1 has roughly even odds of clearing, and irreversible decisions taken on that single result will be wrong about half the time.

Choosing a threshold is choosing which error to make

At 0.1 per cent the assay has 98 per cent specificity and 51 per cent sensitivity; at 0.040 per cent it has 87 and 74 per cent [15]. Neither is

correct in the abstract. If the action triggered by a positive result is allogeneic transplantation, with its associated mortality, high specificity is worth paying for in missed cases. If the action is closer surveillance or a low-toxicity pre-emptive agent, the calculus reverses and the lower threshold is preferable.

The current recommendation applies one threshold to all situations and all assays [2,4], which is convenient for standardisation and difficult to justify clinically. The empirical work points toward assay-specific thresholds, potentially set at the limit of quantification, with the clinical cutoff chosen according to the intended intervention [8,16].

Study limitations

The limitations belong to the underlying literature. Flow cytometric residual disease is operator-dependent and results vary with panel design, gating strategy and laboratory experience, which is why threshold recommendations were made assay-agnostic in the first place [2,4,20]. Comparisons between timepoints derive from cohorts in which timepoints were not randomly assigned [12,14]. The surveillance analysis rests on a case-control design comparing marrows before relapse against marrows in sustained remission, which overstates discrimination relative to unselected serial monitoring [15]. Several cohorts predate current molecular classification, so residual disease is being evaluated against shifting risk categories [7,21]. And immunophenotype can change between diagnosis and relapse, so a phenotype-based assay may miss disease that has evolved [22,23].

Two gaps deserve naming. No randomised trial has compared management guided by a 0.040 per cent threshold against the 0.1 per cent standard, so the case for lowering it rests on accuracy rather than on outcome [8,15]. And no study reviewed here establishes whether pre-emptive treatment initiated on a positive surveillance result improves survival compared with treating at haematological relapse [15,24].

Conclusion

Flow cytometric residual disease predicts relapse in adult acute myeloid leukaemia reliably enough to guide consolidation and transplant decisions, with an 80 per cent one-year relapse probability in patients whose remission carries detectable disease and no transplant, against 20 per cent in those whose does not. The 0.1 per cent threshold that defines positivity was a consensus choice, and testing it across 2,051 patients showed it stratifies suboptimally while discarding information below it. Lowering the cutoff to 0.040 per cent raises sensitivity from 51 to 74 per cent at a specificity cost from 98 to 87 per cent, which is the right trade only if the resulting action is low-risk. A negative result is stable between cycles and can be acted on; a positive one persists in barely half of cases and should

be confirmed before anything irreversible follows from it.

Three changes would improve on current practice. Thresholds could be set per assay at the limit of quantification rather than uniformly at a fixed percentage, which is what the empirical work supports [8,16]. The clinical cutoff could then be chosen according to what the result will trigger, higher where the action is transplantation and lower where it is surveillance [15]. And a positive result taken in isolation could be treated as provisional pending confirmation at the next timepoint, given how often it fails to persist [14].

None of these requires a new assay or a new trial to implement, only a change in how an existing measurement is reported and read. What does require a trial is the question underneath all of it: whether acting pre-emptively on a positive surveillance result improves survival compared with treating at haematological relapse, which remains unanswered [15,21].

Acknowledgements

The authors thank the cooperative study groups and the European LeukemiaNet Working Party whose published analyses form the substance of this article.

Funding

No dedicated funding from public, commercial or charitable sources supported this review.

Conflict of Interest

The authors declare no competing interests.

Ethics Statement

This article reviews previously published studies only. No new patient data were collected and no participants were recruited, so ethical approval was not required.

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