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Real-life sensitivity of flow cytometry minimal residual disease assessment for plasma cell neoplasms

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To the Editor:

Multiple myeloma (MM), a malignancy of mature plasma cells, is considered the second most common hematological malignancy in the United States [1]. While recent advances in therapeutic options have significantly prolonged progression-free and overall survival, MM remains an incurable disease [2]. With increasing efficacy of treatments and advancements in technology, the concept of minimal residual disease (MRD) monitoring for MM was introduced in the early 2010s, to describe the presence of disease detectable below the level of microscopy and routine assays performed on the bone marrow and/or blood specimens. Two types of assays became widely accepted for evaluating the presence of residual abnormal plasma cells: DNA-based next-generation sequencing (NGS) assays and high sensitivity flow cytometry (FC) immunophenotyping assays. In 2016, the International Myeloma Working Group (IMWG) published guidelines for MRD testing in MM with the intent to use the term "MRD" for assays with the minimum sensitivity of 10^{-5} [3]. Currently the only FDA approved test for MM MRD is NGS-based clonoSEQ© by Adaptive Biotechnologies, with the sensitivity of 10^{-6} [4, 5]. The most widely used FC method has been developed by the Euroflow consortium, composed by 19 laboratories around the world (often referred to as next-generation flow, NGF); this is a 2-tube, 8-color Conformité Européenne in-vitro diagnostic (CE-IVD) assay with the advertised sensitivity of 10^{-5} [6, 7]. A dry version of the antibody combination panel and the Infinicyt software for analysis and commercialized by Cytognos.

Comparison between NGF and NGS methods have been made in the past [8, 9], and in general both methods are considered adequate for MRD assessment at the current definition of 10^{-5} sensitivity [10]. Each of the techniques has its advantages and limitations. Advantages of NGF include fast turnaround time, no prior requirement of a diagnostic test and lower overall cost, while the main advantages of NGS are higher sensitivity and lack of requirement for a fresh sample. Most of the reported comparative studies have used data obtained from clinical trials, with stringently controlled methods and central review. From an operational standpoint, NGF and NGS MRD assays may be employed essentially interchangeably by individual laboratories, based on other practical considerations, such as instrumentation, specimen volume, and available local expertise.

At our institution, we adopted NGF MRD assay for MM, early in 2017, and have, since, evaluated over 13,000 bone marrow specimens for the presence of MRD in plasma cell neoplasms, including MM. For internal cases, our unique approach involved screening all the bone marrows with a lower-sensitivity assay (PC proliferation assay, PCPRO, a 7-color assay validated for a sensitivity of 4×10^{-5}) [11], with only PCPRO-negative samples being reflexed to the MRD test. We have recently reported that, in treated MM patients, PCPRO successfully detects 75% of the total MRD-positive cases, with approximately 25% of all PCPRO-negative samples showing MRD-positivity by NGF [12].

The important parameters contributing to the sensitivity of a FC assay are (1) the total number of events collected; (2) the minimal number of events to define an abnormal population; and (3) the difference of antigen expression between normal and abnormal populations. All three parameters are clearly described in the SOP for the Euroflow method (NGF), with the recommended systematic acquisition of 10^7 events.

To determine the real-life sensitivity of the FC MRD assay for MM, we retrospectively reviewed cases examined at our institution. During the period 2017–2023, we analyzed 3137 specimens collected internally, representing 1945 unique patients. As mentioned above, we use the lower sensitivity PCPRO test for screening bone marrows before applying the high sensitivity NGF. The median number of collected events was 8.3×10^6 (25th and 75th percentiles 6.3×10^6 and 9.4×10^6, respectively), with the corresponding sensitivity of 2.4×10^{-6} (2.1×10^{-6} and 3.2×10^{-6}). In the same time period, we analyzed 10452 external (sent-in) specimens from 142 different clinics and hospitals in the United States. All external specimens were analyzed within the validated time (up to 72 hours from collection). The median number of collected events for external specimens was 7.0×10^6 (25th and 75th percentiles 4.7×10^6 and 8.6×10^6, respectively), with the corresponding sensitivity of 2.8×10^{-6} (2.3×10^{-6} and 4.2×10^{-6}) (Table 1).

Internally collected PCPRO-negative cases reflexed to FC MRD assay showed that 2380 out of 3137 cases (75.9%) were MRD-negative, 19 (0.6%) were MRD-indeterminate (subjectively assessed due to either <20 clonal events in a cluster, or ≥20 events but with unconvincing phenotype and/or clustering), and 738 (23.5%) were MRD-positive. As a comparison, external (not pre-screened) specimens showed increased percentage of MRD-positive specimens (43.3%), with 15.7% of them showing large clones (above 0.1% of total events), in comparison to only 0.3% of internal cases. There was no significant difference in parameters of hemodilution (mast cell and hematogone percentages) between internal and external cases (Table 1).

Several important conclusions can be made from the data presented here. First, FC assessment of MRD in plasma cell neoplasms is feasible even in reference laboratory setting, if the sample is analyzed within the validated analytic timeframe (72 h for our laboratory). Second, the real-life (non-clinical trial-associated) sensitivity of FC MRD test for plasma cell neoplasms is better than the IMWG-defined minimal sensitivity of 10^{-5} and is closer to the sensitivity of the NGS assay (10^{-6}). No direct comparison is made between NGS and NGF in this data set. Finally, pre-screening of bone marrow aspirates with a lower sensitivity assay (PCPRO) helps optimal MRD test utilization by excluding specimens with high level involvement by clonal plasma cells.

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Table 1. Comparison of FC MRD assay performance between specimens collected internally (in-house) and specimens collected at other institutions (external, sent-in).

	Internal	External (sent-in)	
Total cases analyzed	3137	10452	
Median events collected (25th and 75th percentiles)	8.3×10^6 (6.3×10^6 and 9.4×10^6)	7.0×10^6 (4.7×10^6 and 8.6×10^6)	*$p < 0.0001$
Median sensitivity (25th and 75th percentiles)	2.4×10^{-6} (2.1×10^{-6} and 3.2×10^{-6})	2.8×10^{-6} (2.3×10^{-6} and 4.2×10^{-6})	*$p < 0.0001$
Median % mast cells (25th and 75th percentiles)[a]	0.011 (0.004 and 0.031)	0.012 (0.004 and 0.038)	*$p = 0.1953$
Median hematogones (25th and 75th percentiles)[a]	0.511 (0.096 and 1.782)	0.639 (0.103 and 2.073)	*$p = 0.1679$
Percent MRD-positive cases	23.5	43.3	**$p < 0.0001$
Percent MRD-positive cases above 0.1% of total events (high positives)	0.3	15.7	**$p < 0.0001$

*Mann-Whitney U test (Wilcoxon rank-sum).
**Z-score for population proportions.
[a]from the subset of more recent data (last 620 of internal and 2602 of external cases).

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AUTHOR CONTRIBUTIONS

DJ, SKK, and HO designed the study; DJ and GEO analyzed the data; DJ wrote the draft of the letter; MS, PH, GEO, MMT, LBB, PTG, WIG, PK, MAG, MB, FKB, JZ, AD, TK, EM, SVM, SKK, and HO critically reviewed the data and the letter.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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