FALSE NEGATIVE RATE OF MINIMAL RESIDUAL DISEASE MEASUREMENT BY MULTIPARAMETER FLOW CYTOMETRY IN MULTIPLE MYELOMA WHEN COMPARED TO TREPHINE IMMUNOHISTOCHEMISTRY

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Context

- Minimal residual disease (MRD) monitoring with multiparameter flow cytometry (MFC) is an increasingly important prognostic tool in treated multiple myeloma (MM) and may become a surrogate endpoint for clinical trials
- MFC underestimates MM plasma cell percentage (MMPC%) when compared to trephine and false MRD- may occur due to various factors such as nodular MM distribution, marrow fibrosis and aspirate haemodilution
- There is little published data on actual false MRD- rates by MFC
- We aimed to estimate the rate of false MRD- when compared to trephine immunohistochemistry (IHC)

Materials and methods

- 754 paired bone marrow aspirate and trephine samples from 368 MM patients obtained as per local protocol before and after autologous stem cell transplantation at the Royal Marsden Hospital (London, UK) between 2011 and 2015 were retrospectively compared
- MFC was performed with an 8-colour panel; $5x10^5$ cells were acquired and MRD- was defined as less than 0.01% MMPCs. Results were compared with MMPC% from trephine IHC
- Correlation and agreement between the paired results were assessed with Spearman's rank-order and Passing-Bablok regression
- The false MRD- rate by MFC was calculated using a "true-positive" standard of ≥5% MMPCs by trephine IHC

Results

Fig. 1: Passing-Bablok non-linear regression MFC vs IHC

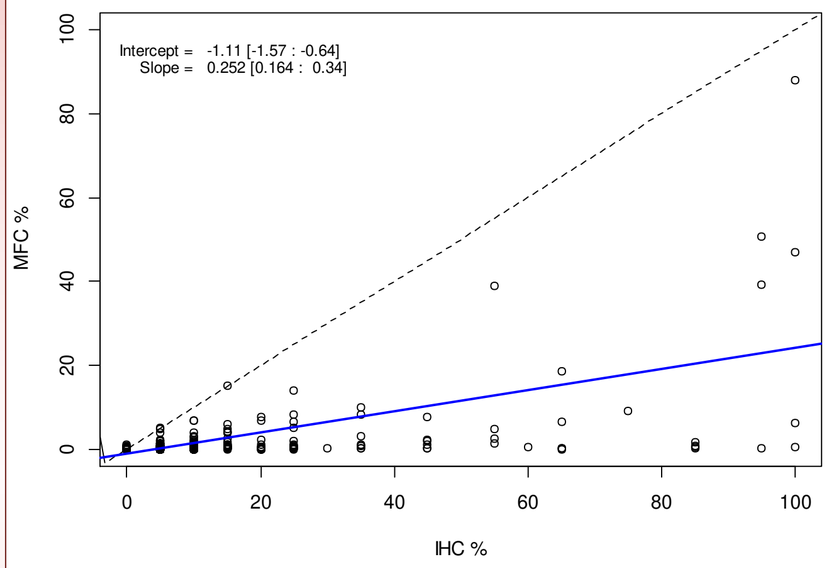
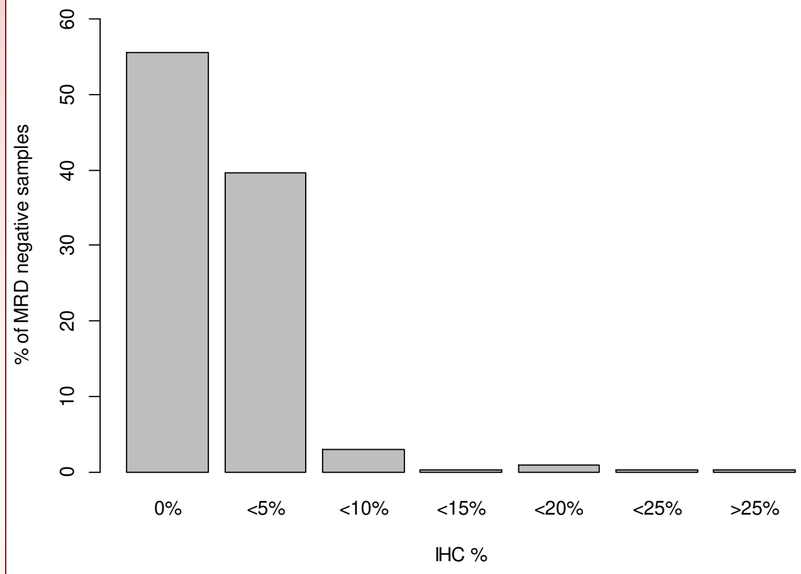


Fig. 2: Distribution of IHC % results for MRD negative samples



- A positive correlation between MMPC% by IHC and MFC was observed, ρ=0.58, p<0.001
- Non-linear regression, shown in fig. 1, showed fixed and proportional underestimation bias for MFC
- Fig. 2 shows the distribution of IHC results amongst the 331 MRD- samples: 55.6% had 0% MMPC by IHC, 39.6% had detectable MMPC at a level <5%
- 4.8% of MRD- samples had MMPCs detectable above 5% by IHC

	IHC ≥ 5%	IHC < 5%
MRD+	140	283
MRD-	16	315

- The above confusion matrix shows the numbers of samples by corresponding MFC and IHC results
- For MFC with sensitivity 0.01%:

  FALSE NEGATIVE RATE = 10.3% [5.5 – 15.1]

  NEGATIVE PREDICTIVE VALUE = 95.2% [92.9 – 97.5]

Summary

- We observed a positive correlation between MMPC% calculated with aspirate MFC and trephine IHC
- The underestimation bias of MFC compared to IHC is most likely to be explained by aspirate haemodilution
- The majority of MRD- samples had either no detectable MMPCs by IHC or detectable at a level below 5%
- The false negative rate was 10.3% and the negative predictive value was 95.2% in the sample in which the MRD negativity cut-off was 0.01%
- The IMWG suggests MRD negativity should be defined below 0.001% and using this standard the false negative rate observed here should be reduced
- MRD measured by aspirate MFC should be interpreted with other tests, such as trephine IHC, for quality assurance purposes