CLINICAL IMPACT OF THE EUROPEAN MYELOMA NETWORK GUIDELINES FOR MULTIPARAMETRIC FLOW CYTOMETRY (MFC) ANALYSIS OF MYELOMA PLASMA CELLS - REPORT FROM THE NMSG #9/99 PROJECT

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Background Recently, the European Myeloma Network (EMN) has published guidelines for MFC in MM, which we have applied and here we present results from analysis of 280 bone marrow (BM) samples from teen Nordic centres that support a diagnostic, prognostic and predictive impact in clinical practice.

Design and Methods This registration study were planned to include a minimum of 100 diagnostic samples analysed in accordance with the EMN guidelines for MFC with the aims to evaluate the number and the phenotypic profile of myeloma plasma cells (PC) and the impact of minimal residual disease. Samples were analysed as described previously for identification of myeloma end stage CD45/CD38++ PC. Registration included relative numbers of events and calculated mean fluorescence intensity (MFI) for B cell membrane antigens and auto-fluorescence within the PC gate. All MFI ratios were calculated for cluster analysis performed with software by the hierarchical ordering of Pearson's coefficients method.

Results First, the median MFC-PC number was 15% and LM-PC 35% for corresponding samples significantly correlated (Figure 1a & b). The difference between MFC- and LM-PC numbers *at diagnosis* was significantly influenced only by the CD56 MFI on PC, indicating that homophile cell-cell binding may explain the difference. At least 10% plasma cells in bone marrow aspirate counted by light microscopy is a diagnostic demand and therefore we studied the variation of the MFC-PC number in the planned 80 MM and 20 MGUS patients as illustrated in Figure 1a. The distributions indicate that a cut off value detected by MFC of 1 % PC (Figure 2a) discriminate MM disease and premalignant MGUS samples with a sensitivity of 0.83 (95% C.I. 0.75-0.92) and specificity of 0.53 (95% C.I. 0.30-0.75) respectively. Such a discriminatory value is based on the dataset shown in Figure 2b from analysis of 67 non diagnostic BM samples received during the study period. These samples were aspirated due to a clinical need for PC enumeration for evaluation of treatment outcome. These data therefore reflects daily decision taking for BM analysis monitoring patients influenced by symptoms, organ involvement or the level of M component and seem to divide patients in two clear groups by MFC-PC number of 1%. These results are in support for inclusion of plasma cell number by MFC into the diagnostic triad for MM – but needs to be prospectively validated.

Second, hierarchical unsupervised clustering of MFI revealed a patient cluster I with high membrane intensity for CD56, CD38, CD45, SSC, FSC, and low for CD28, CD19 and a patient cluster II, (N = 50) with low intensity of CD56, CD38, CD45, SSC, FSC and high for CD28, CD19 (Figure 3a). The *prognostic impact* of such clusters revealed a median survival of 39 and 19 months respectively (P=0.02) (Figure 3b).

Third, enumeration of minimal residual disease in bone marrow aspirates from 16 high dose melphalan treated patients revealed a reduction of MFC-PC from median 1.7% at diagnosis to 0.22% and 0.16% following 1st and 2nd transplantation (Figure 4a). A further decreased level 2-3 months after the 2nd HDT *predicted superior* EFS (Figure 4b).

Conclusions The guidelines for MFC in MM do produce diagnostic, prognostic and predictive information useful in clinical practice, which however needs to be prospectively validated as planned within EMN.

Reference

Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. Rawstron AC, Orfao A, Beksac M, Bezdickova L, Brooimans RA, Bumbea H, Dalva K, Fuhler G, Gratama J, Hose D, Kovarova L, Lioznov M, Mateo G, Morilla R, Mylin AK, Omedé P, Pellat Deceunynck C, Perez Andres M, Petrucci M, Ruggeri M, Rymkiewicz G, Schmitz A, Schreder M, Seynaeve C, Spacek M, de Tute RM, Van Valckenborgh E, Weston-Bell N, Owen RG, San Miguel JF, Sonneveld P, Johnsen HE; European Myeloma Network. Haematologica. 2008 Mar;93(3):431-8.

FIGURE 1a

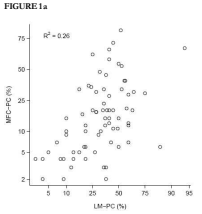

Figure 1a legend Comparison of logit transformed (i.e. log(p/(1-p)) plasma cell percentage by MFC (MFC-PC) and by light microscopy (LM-PC) significantly correlated but is heavy with MFC-PC numbers 3 - 8 times corresponding LM-PC number suggesting selective loss of events during flow analysis compared to smear analysis.

FIGURE 1b

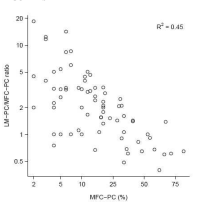

Figure 1b legend Comparison of ratio LM-PC/MFC-PC and the plasma cell percentage enumerated by MFC negative correlated. No correlation was identified when compared to plasma cell percentage enumerated by LM (not shown).

FIGURE 2a

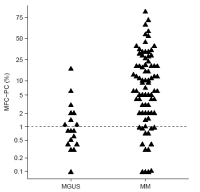

Figure 2a legend Analytic plot illustrating the variation of the MFC-PC numbers in 20 MGUS and 80 MM patients analysed (1 MGUS and 2 MM are excluded due to technical reasons). The distribution indicate that a cut off value detected by MFC of 1 % PC discriminate samples from MM disease and the premalignant stage MGUS with a sensitivity of 0.83 (95 % C.I. 0.75-0.92) and specificity of 0.53 (95% C.I. 0.30-0.75).

FIGURE 2b

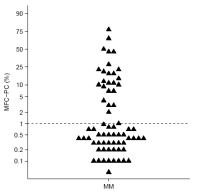

Figure 2b legend Analytic plot illustrating MFC-PC numbers in 67 non diagnostic BM samples received during the study period shown above. The sample was aspirated for analysis due to a clinical need for PC enumeration in a follow up of treated patients with MM. This indicates a discriminatory cut off value detected by MFC of 1 % PC.

FIGURE 3a

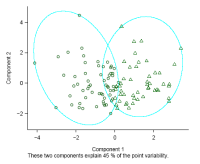

Figure 3a legend Unsupervised principal component analysis (PCA) of the data set illustrating the two cluster I (N=50) and II (N=53) based on MFI CD/MFI auto fluorescence ratios for the subsets within the gated plasma cell compartment. The figure also illustrates a minor third group of patient who are poorly classified (N=14), see Figure 3b).

FIGURE 3b

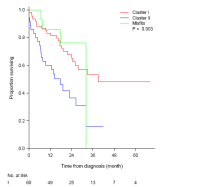

Figure 3b legend Survival for Cluster II and with median survival of 39 months and for Cluster II (blue) of 19 months and 4 months (green). 28 months, significant different (P=0.008).

FIGURE 4a

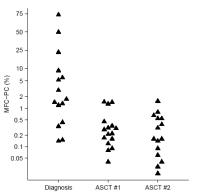

Figure 4a legend Estimation of minimal residual disease by the MFC numbers (MFC-PC%) in bone marrow samples from 16 patient treated with high dose melphalan and autologous stem cell transplantation. A significant reduction from median 1.7% (range 1-30 %) at diagnosis to 0.22% (range 0.02-2 %) and 0.16% (range 0-2 %) after 1st and 2nd transplantation, respectively.

FIGURE 4b

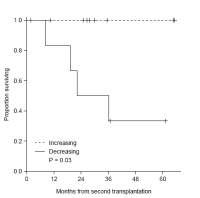

Figure 4b legend A decreased PC level following 2nd HDT resulted in a 100% event free survival (green) which is significant better than for patients with an increasing PC level 2-3 months after 2nd high dose melphalan supported by autologous stem cell transplantation (blue).