European Congress of Clinical Chemistry and Laboratory Medicine

Paris, France – Le Palais des congrès 21-25 June 2015

Automated Pleural and Ascitic Fluids Cell Counting on Mindray BC-6800

S.Buoro, T. Mecca, G. Azzarà, M. Seghezzi, M. Vavassori, A.Crippa, A. La Gioia

Chimical Chemistry Laboratory A. O. Papa Giovanni XXIII – Piazza OMS, 1 - 24128 Bergamo Italy

INTRODUCTION

The study of body fluids (BFs) is of great clinical importance and is often required in hospital settings as an urgent procedure. Many of the automated hematology analyzers nowadays available offer an integrated platform specifically designed to give the advantages of both rapidity and standardization of BF analysis. The purpose of this study was to evaluate the application of Mindray BC-6800 BF-Mode in cytometric analysis of pleural (PF) and ascitic fluids (AF), according to the International Cut-offs recommended by CLSI H56-A guideline(1), i.e. Nucleated Cells (NC) $\geq 1000*10^6$cells/L with Polymorphonuclear (PMN) >50% or Lymphocytes cell count >50% (for diagnosing acute inflammation, infection or tubercular metastasis, lymphoproliferative disorders and chylous effusions) and PMN > $250*10^6$cells/L in AF (for spontaneous bacterial peritonitis).

MATERIALS AND METHODS

A total of 118 consecutive fresh samples of BFs (88 AF and 30 PF) collected in K_3EDTA tubes and with total cellularity range between 11 to $8733*10^6$cells/L were analyzed without pre-treatment using the BC-6800 BF-Mode and then underwent a full Optical Microscope (OM) examination. Nucleated Cell Count (NC) in Nageotte chamber and morphological classification after cytocentrifugation and May-Grunwald-Giemsa staining (2,3) were performed at OM. Finally, the correlation between the above mentioned methods was assessed by Pearson's coefficient, Passing-Bablok regression and Bland-Altman bias. Diagnostic accuracy was determined with ROC curve analysis. The statistical analysis was carried out with Analyse-it™ Software (Ltd. Leeds, UK).

Body Fluid
- Ascitic
- Pleural
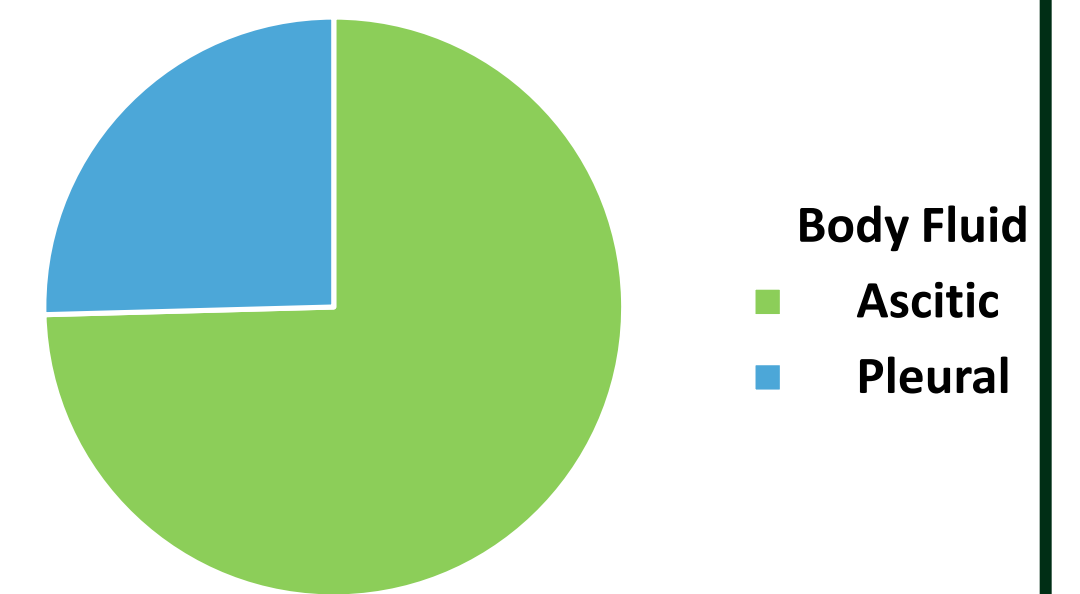
RESULTS

The BC-6800 BF-Mode, compared to OM, as for NC, PMN and Mononucleated (MN) cell counts showed a Person's correlation respectively of: r=0.99, r=0,98 and r=0.96 (always with p<0.001); a Passing and Bablok regression: y=1.04X+0.77, y=1.01x+11.29, y=1.13x-22.97 and a Bland Altman Bias of 31.7, 6.7, 78, for each of the above mentioned cell types (Figure 1, 2, 3 and table 1).
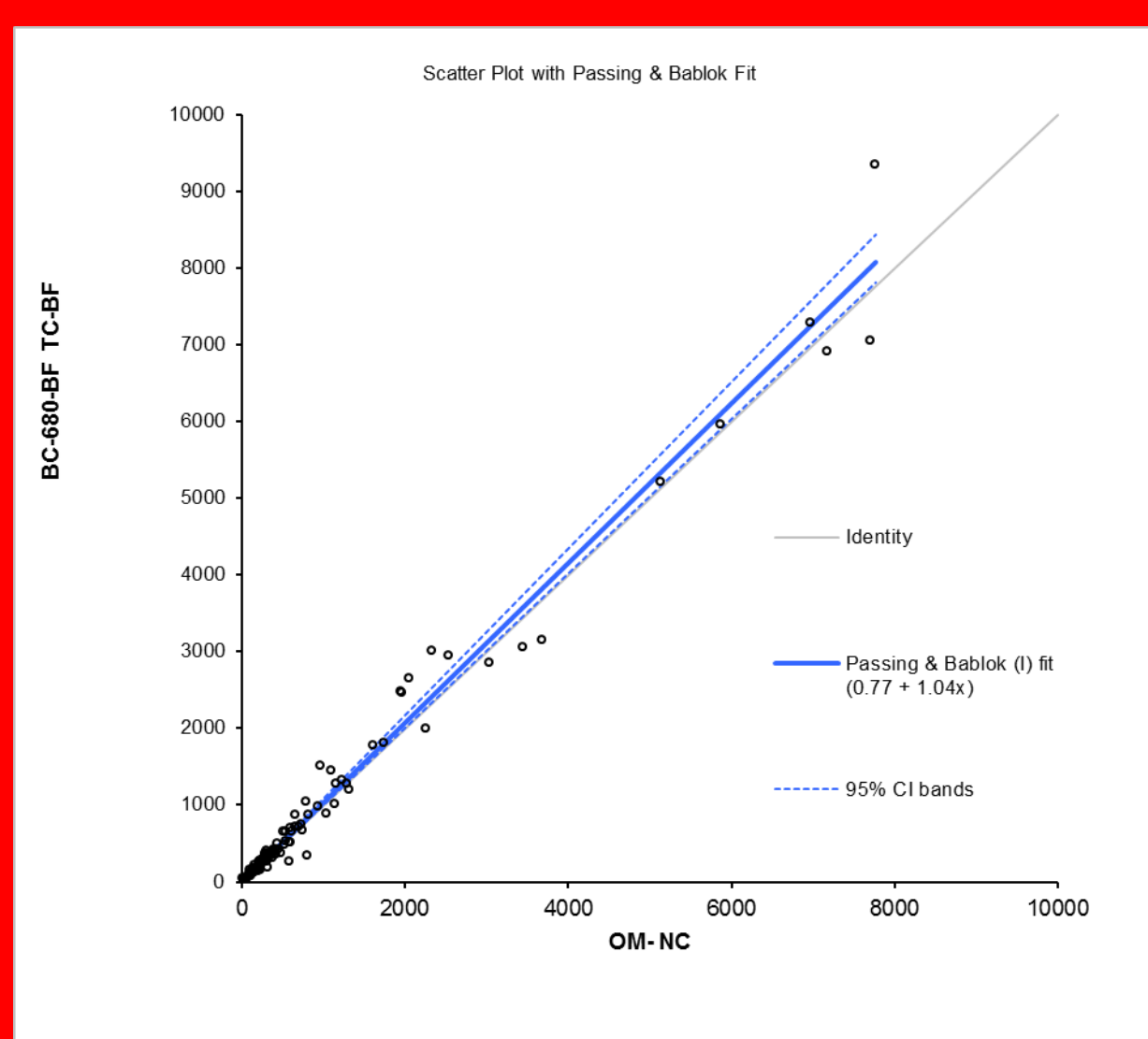

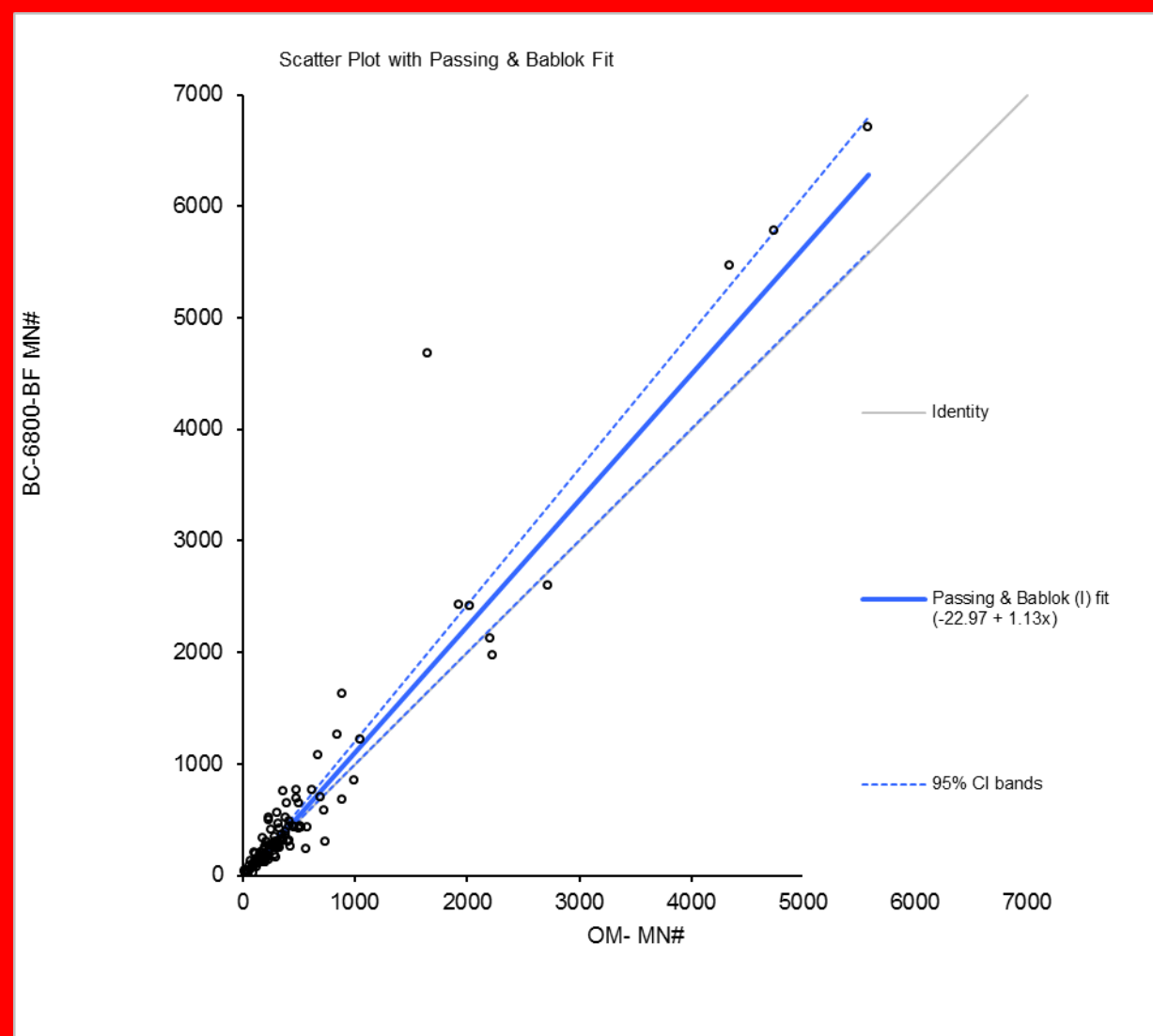Figure 1: Passing Bablok regression for Nucleated Cell Count

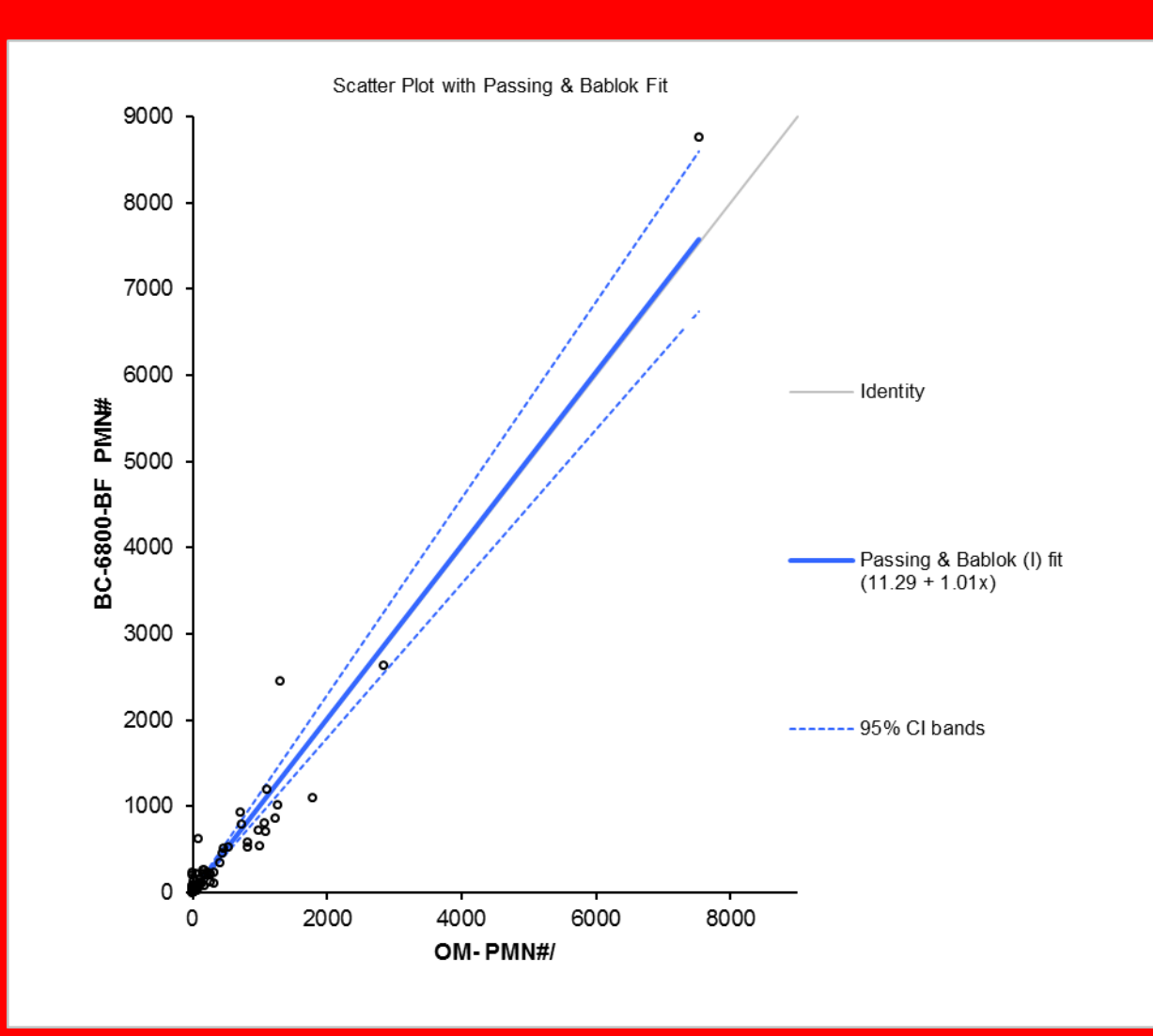Figure 2: Passing Bablok regression for Mononucleated Cells Count

Figure 3: Passing Bablok regression for Polymophonuclear Cell Count

Table 1: Pearson's correlation, Passing Bablok regression and Bland Altman Bias for NC, PMN and MN

	Pearson correlation (r) (p value)	Passing-Bablok regression (CI 95% Slope and Intercept)	Bias Altman-Bland (CI 95%)
NC-BF	0.99 p<0.0001	y=1.04X+0.77 (Slope : 1.01 to 1.09 Intercept : -11.08 to 14.05)	31.7 (-5.1 to 79.3)
PMN-BF	0.98 p<0.0001	y= 1.01x+11.29 (Slope : 0.89 to 1.14 Intercept: 7.99 to 17.08)	6.7 (-33.8 to 47.2)
MN-BF	0.96 p<0.0001	y=1.13x-22.97 (Slope : 1.00 to 1.22 Intercept: -40,43 to 1,07)	78.0 (13,0 to 143,2)

The ROC curve analysis of PMN absolute count in AF showed an area under curve (AUC) of 0.99 and the Diagnostic Agreement obtained was 95% at the cut-off of PMN>250 cells/µL. The ROC curve analysis of PMN% count in PF showed an AUC of 0.91 and the Diagnostic Agreement obtained was 83% at the PMN cut-off>50% (Table 2 and Figure 4)
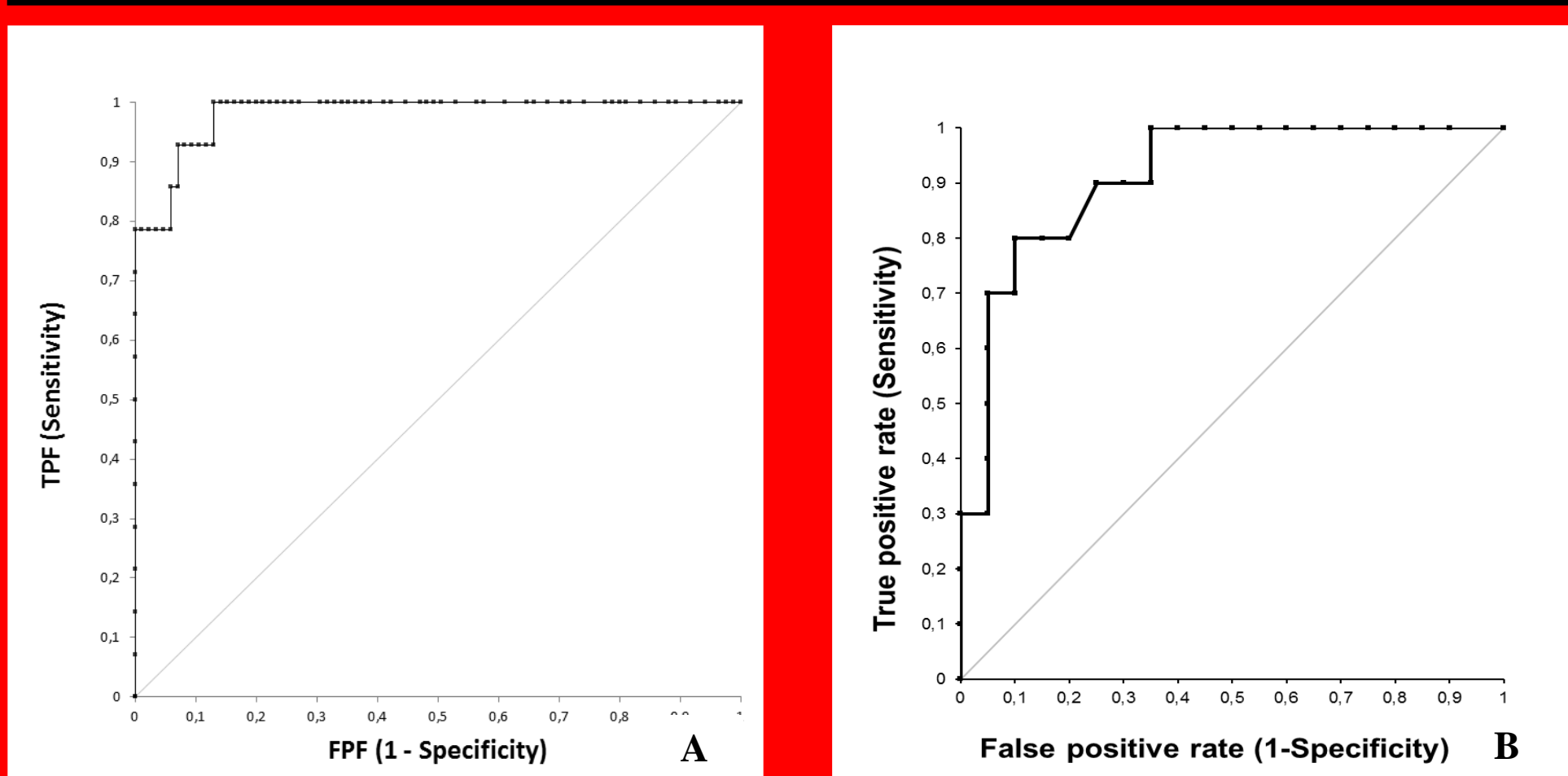

Figure 4: ROC Curves for (A) PMN# in Ascitic Fluids and (B) PMN% Pleural Fluids

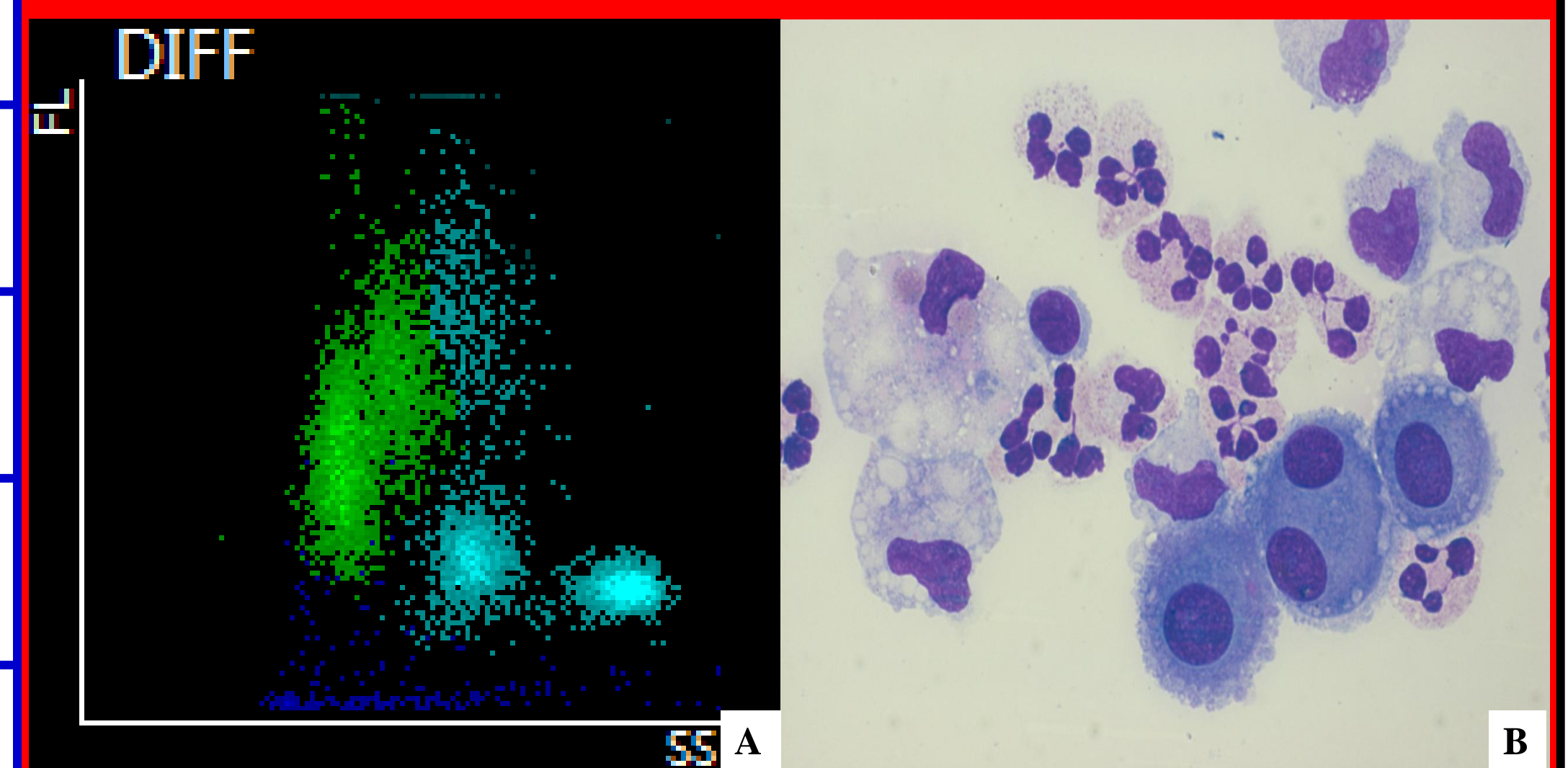|	AUC (CI 95%) p-value	CUT-OFF	Diagnostic Agreement
PMN # (Ascitic Fluid)	0.99 (0.96 to 1.00) p<0.0001	≥250 x10^6cells/L	95% (1 false negative and 3 false positive)
PMN % (Pleural Fluid)	0.91 (0.81 to 1.00) p<0.0001	≥50%	83% (1 false negative and 4 false positive)

Figure 5: (**A**) Abnormal DIFF Scattergram due to a misclassification of Neutrophils caused by the presence of Macrophages confirmed in optical microscopy (OM)(**B**). **Mindray BC-6800:** TC-BF: $1642*10^6$cells/L; PMN: 43.6%; MN: 56.4%L **OM:** neutrophils 41%; lymphocytes 19%; macrophages 7%; mesothelial cells 5%; eosinophils 18% and OM-TC $1587*10^6$cells/L.
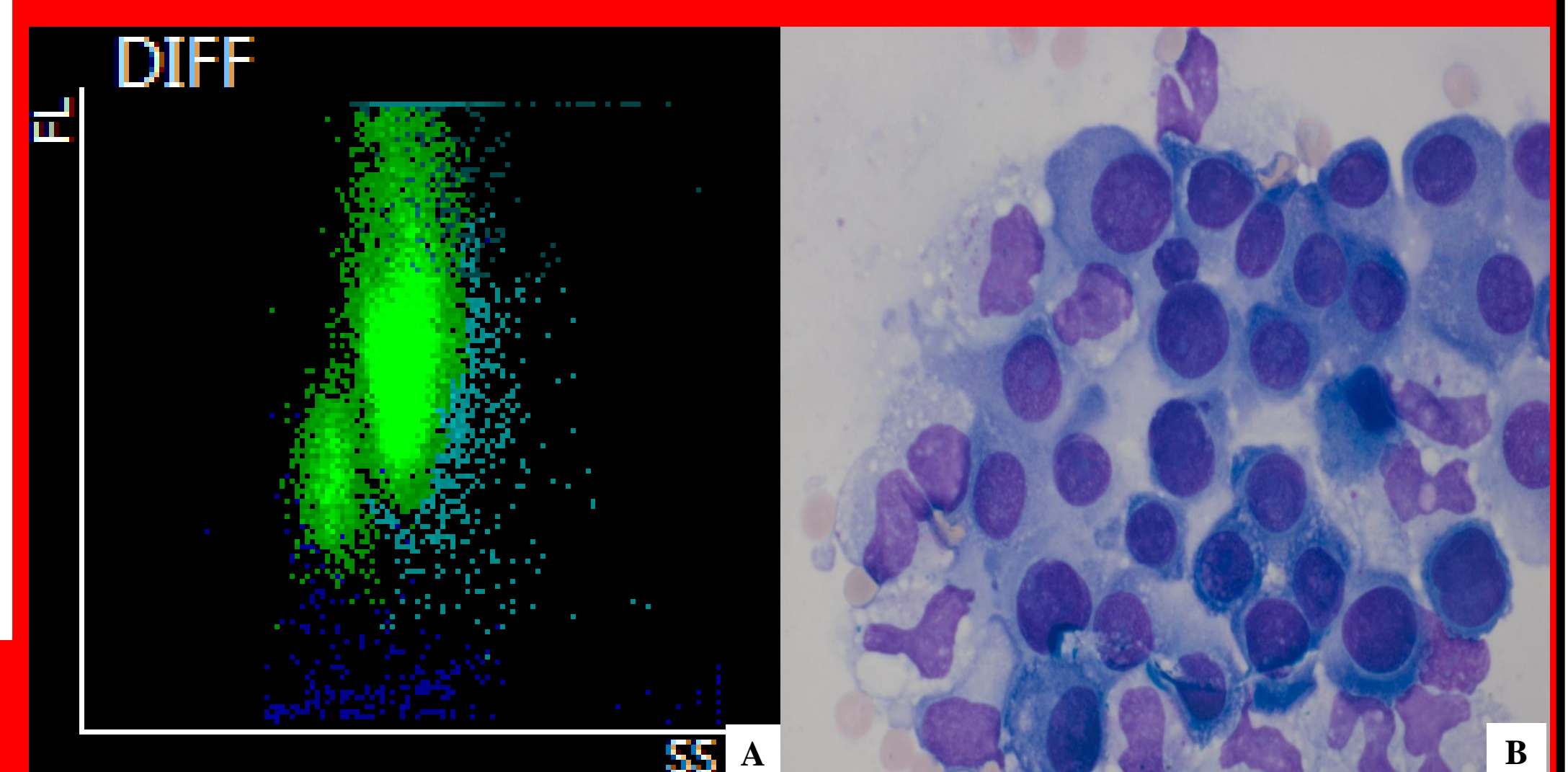

Figure 6: (**A**) Abnormal DIFF Scattergram due to the precence of High Fluorescence cells (HF). (**B**) Morphological analysis with OM reveals the presence of atypical cells recognized like tumoral cells **Mindray BC-6800:** TC-BF: $5220*10^6$cells/L; PMN: 4.2%; MN: 95.8%L **OM:** neutrophils 1%; lymphocytes 2%; monocytes 71; other neoplastic cells 26% and OM-NC $5133*10^6$cells/L.

CONCLUSIONS

Although OM analysis is still considered the reference method for cellular analysis of BF samples, automated procedures that could replace the manual ones are looked by many laboratories as the best solution to provide clinicians with cost-effective, rapid and accurate results.
Our study demonstrated the utility of BC-6800 in Cell Count and Differentiation of AF and PFs in automated BF-Mode.
BC-6800 in AF and PF offers rapid and accurate measures in clinically relevant cellularity ranges.
The use of BC-6800 in BF-Mode may offer the opportunity to replace routine optical counting, with the exception of samples displaying abnormal cell counts or DIFF scattergrams as shown in Figure 5 and 6.

REFERENCES

1. Body Fluid Analysis for Cellular Composition; Approved Guidelines. CLSI H56-A;
2. Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of instrumental Methods - Approved Standard, CLSI H20A2;
3. ICSH guidelines for the evaluation of blood cell analyzers including those used for differential leucocyte and reticulocyte counting, Lab Hem.