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Impact of aspirate smears and trephine biopsies in routine bone marrow diagnostics: a comparative study of 141 cases

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Summary

The diagnostic impact of bone marrow cytology in combination with flow cytometry analysis of aspirate smears and bone marrow histology together with immunohistochemical examination of trephine biopsies was compared in 141 routine cases. Diagnoses achieved by the two methods were concordant in 80.5% of cases. In discordant cases, clinical follow-up data of at least one year confirmed the correctness of cytological and histological diagnoses. For infiltration by malignant disease, both methods were concordant in 86.5% of samples and correlated well for the degree of infiltration ($r = 0.64$, $p < 0.001$). Overall, regression analysis showed a good correlation for cellularity ($r = 0.67$) lymphopoiesis ($r = 0.75$), granulopoiesis ($r = 0.73$) and megakaryopoiesis ($r = 0.65$) while erythropoiesis displayed a lower degree of correlation ($r = 0.43$, all $p < 0.001$). Regression analysis on all immunological data obtained by flow cytometry (FC) and immunohistochemistry (IHC) showed a good overall linear correlation ($r = 0.67$, $p < 0.001$), but significant differences were found for a few phenotypic markers. Furthermore, the correlation was found to be dependent on IgG subclasses and the fluorochromes used for FC. Thus, analyses with IgG_2 antibodies and phycoerythrin (PE) as fluorochrome showed significantly more expression than IHC. In conclusion, cytology and histology, both in association with the respective immunophenotyping, are of equal value in bone marrow diagnostics and should be used in combination. However, in some specific settings, one of the two procedures might be preferable.

Key words: aspirate smears; trephine biopsy; flow cytometry; immunohistochemistry; diagnostics

Introduction

In Anglo-American countries bone marrow diagnostics are completely in the hands of haematopathologists, whereas in continental Europe aspirate smears are often examined by haematologists, while trephine biopsies are handled by pathologists. This is also the case at the cantonal hospital in St.Gallen, Switzerland, a tertiary medical centre. In 1997, an interdisciplinary bone marrow board consisting of pathologists, haematologists and oncologists was established on a weekly basis. Cases were selected if they required either a mutual consensus or were of a special interest to the participants.

The present study compares the diagnostic impact of aspirate smears and trephine biopsies as they are routinely processed and analysed morphologically and immunophenotypically by haematologists and pathologists. Current knowledge indicates that both procedures have advantages and disadvantages in establishing the final diagnosis [1–5]. However, a combined approach is mandatory in order to optimise bone marrow diagnostics in process and outcome [6, 7].

Patients and methods

One hundred and forty-one samples from 132 patients as discussed on the weekly bone marrow board between 1998 and 2002 were studied.

Bone marrow aspirates and trephine biopsies

All samples were obtained through bone marrow (BM) sampling (aspirate and biopsy) from one of the pos-

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Table 1

Specifications of antibodies used for immunophenotyping with the PAP method (TdT = terminal deoxynucleotidyl transferase).

epitope	isotype	clone	species	company
CD45	IgG_1	2B11 + PD7/26	mouse	DakoCytomation®
CD20	IgG_2a	L26	mouse	DakoCytomation®
CD10	IgG_1	56C6	mouse	DakoCytomation®
CD23	IgG_1	1B12	mouse	Novocastra®
IgM		polyclonal	rabbit	DakoCytomation®
κ-light chains		polyclonal	rabbit	DakoCytomation®
γ-light chains		polyclonal	rabbit	DakoCytomation®
CD3		polyclonal	rabbit	DakoCytomation®
CD4	IgG_1	1F6	mouse	Novocastra®
CD5	IgG_1	4C7	mouse	Novocastra®
CD7	IgG_1	CD7-272	mouse	Novocastra®
CD8	IgG_1	C8/144B	mouse	DakoCytomation®
myeloperoxidase		polyclonal	rabbit	DakoCytomation®
glycophorin A	IgG_1	JC159	mouse	DakoCytomation®
CD34	IgG_1	Q-Bend 10	mouse	Immunotech®
TdT		polyclonal	rabbit	DakoCytomation®

Table 2

Specifications of antibodies used for immunophenotyping with flow cytometry (FITC = fluorescein isothiocyanate, PE = phycoerythrin).

epitope	fluorochrome	isotype	clone	species	company
CD45	FITC	IgG_1	2D1	mouse	BD Biosciences®
CD20	FITC	IgG_1	L27	mouse	BD Biosciences®
CD10	FITC	IgG_2a	W8E7	mouse	BD Biosciences®
CD23	FITC	IgG_1	MHM6	mouse	DakoCytomation®
IgM	FITC	IgM, affinity-isolated F(ab')2	polyclonal	rabbit	DakoCytomation®
κ-light chains	FITC	IgG_1	TB28-2	mouse	BD Biosciences®
γ-light chains	PE	IgG_1	1-155-2	mouse	BD Biosciences®
CD3	FITC	IgG_1	SK7	mouse	BD Biosciences®
CD4	PE	IgG_1	SK3	mouse	BD Biosciences®
CD5	PE	IgG_2a	L17F12	mouse	BD Biosciences®
CD7	FITC	IgG_2a	4H9	mouse	BD Biosciences®
CD8	PE	IgG_1	SK1	mouse	BD Biosciences®
myeloperoxidase	FITC	IgG_1	5B8	mouse	BD Biosciences®
glycophorin A	PE	IgG_1	JC159	mouse	DakoCytomation®
CD34	PE	IgG_1	8G12	mouse	BD Biosciences®
TdT	FITC	IgG_1	HT-6	mouse	DakoCytomation®

terior superior iliac crests using standard procedures [8, 10].

Biopsies

Marrow cores were processed according to standard procedures [8]. 2 µm thick paraffin sections were routinely stained with haematoxylin-eosin (H&E), Giemsa, periodic acid-Schiff reaction (PAS) and Gomori's silver impregnation. Immunostaining was performed with the peroxidase anti-peroxidase (PAP) method [9] using commercially available anti-human antibodies as listed in table 1.

Aspirates

Smears were prepared according to standard procedures [10] and routinely stained by May-Grünwald-Giemsa technique. For immunophenotyping by flow cytometry, aspirates were again prepared according to standard procedures or the recommendations of the manufacturer. All antibodies used were anti-human antibodies as specified in table 2. Flow cytometry (FC) was performed on a FACS Calibur™ (BD Biosciences®). Ten thousand events were counted from each tube and cells were immunophenotyped by 3-colour cytometry. Gating was done as appropriate, usually after displaying the events in a CD45/side scatter plot. As is usual in routine FC, isotype-matched controls were used for each immunoglobulin class and fluorochrome.

Study design

Cytological diagnostics on aspirate smears including flow cytometric analyses and histological diagnostics on trephine biopsies including immunohistochemical examinations were independently performed by haematologists and pathologists, respectively, during routine work-up before cases were discussed on the weekly board. Adequate clinical information was available to all investigators. Stratified in six categories, these diagnoses were then compared with regard to compatibility or incompatibility:

- Lymphoproliferative diseases
- Acute leukaemias, subtyped as acute lymphoblastic leukaemia (ALL) and acute myelogenous leukaemias (AML)

- Myelodysplastic syndromes (MDS)
- Myeloproliferative syndromes (MPS)
- Non-malignant alterations and normal BM
- Metastases of solid malignancies

For compatibility purpose, subtyping was only required for lymphomas (according to the WHO classification [11]) but not for ALL, AML, MDS and MPS. To distinguish between MDS and AML, the FAB classification [12] was used.

In discordant cases, follow-up re-examinations, available cytogenetic and molecular pathological data and clinical outcome over one year of time helped to define the correct diagnosis.

Morphologic evaluation

For study purposes, all aspirate smears were reassessed by a haematologist (WK) and independently all biopsies were reassessed by a pathologist (SBC). It is important to mention that the initial diagnoses were not altered during this reassessment process. The purpose was to semi-quantify samples with regard to cellularity, granulopoiesis, megakaryopoiesis, lymphopoiesis, erythropoiesis and the amount of blasts. Cellularity was graded as hypocellular (–1), normocellular (0) or hypercellular (+1). Granulopoiesis, megakaryopoiesis, lymphopoiesis and erythropoiesis were scored as decreased (–1), normal (0) or increased (+1). Blasts were registered as increased (+1) or not increased (0). In samples with tumour involvement, infiltration-grades were scored as 1 (<10%), 2 (10–30%), 3 (30–50%), 4 (50–70%), or 5 (>70%).

Immunological evaluation

Phenotypic profiling as evaluated by FC on aspirates and by immunohistochemistry (IHC) on trephine biopsies included antibodies against CD45, CD20, CD10, CD23, IgM, κ- and λ-light chains, CD3, CD4, CD5, CD7, CD8, myeloperoxidase (MPOX), glycophorin A (GLY-A), CD34 and terminal deoxynucleotidyl transferase (TdT) antigens. Since exact quantification is not possible in a routine setting by IHC, immunoreactivities were semi-quantified for comparability. Antigen expression in tumour cells (with neoplastic cells gated in FC or morphologically identified in IHC) was stratified as 0 (<10%), 1 (10–30%), 2 (30–80%), or 3 (>80%). Overall, immunoreactivity was scored as positive (expression ≥30% in FC or IHC) or negative (expression <30% in FC or IHC).

Statistical methods

Regression analysis was used to correlate parameters from cytology and histology and the results from flow cytometry and immunohistochemistry, respectively. Furthermore, possible differences in immunophenotyping with regard to the IgG subclasses and the fluorochromes used for flow cytometric analysis were examined with the Wilcoxon paired sample test.

Results

Diagnosis

One hundred and forty-one samples from 132 patients were reviewed. Diagnoses were assigned by both cytology and histology in 133 cases (94.3%, table 3). Eight samples (2 biopsies and 6 aspirates) were excluded due to inadequate material. The diagnoses of aspirate smears and biopsy sections were concordant in 107 of 133 cases (80.5%). In 17 of 26 discordant cases (65%), incongruence was due to the presence or absence of pathological features, while nine cases (35%) were discordant because of evidence of two different pathologies. In 26 discordant cases clinical follow-up data favoured the cytologic or the histological diagnosis in 50 and 50 per cent, respectively.

Lymphoma

Lymphomas were concordantly diagnosed in 38 of 50 cases (76%), (table 4). In only one case discordance was due to different subtyping by the two experts, while the other 11 samples were discordant due to the fact that infiltration was detected by one method only.

In one of 13 samples of small lymphocytic lymphoma/chronic lymphocytic leukaemia (SLL/CLL) bone marrow infiltration was detected by histology only (extent of infiltration <10%). However, in this case no FC was performed.

Cytological and histological interpretation mismatched in four of 8 cases of follicular lymphoma. In two cases with a typical paratrabecular infiltration pattern involving less than 10% of the BM the tumour was only detected by histology and IHC. However, in another two cases also of low grade infiltration (<10%) it was only found by cytology and FC. In one of five cases with hairy cell leukaemia, the diagnosis was made by immunohistology, whereas cytology with FC was not conclusive.

In plasma cell myelomas, cytology and histology were discordant in three of 10 samples (30%). In one case, cytology and FC data favoured a lymphoplasmacytic lymphoma while in two other cases (with an infiltration of <30%) the tumour was only diagnosed by trephine biopsy examination.

Diagnoses were discordant in two of five samples of diffuse large B-cell lymphoma. In one case, BM involvement was only found by histology (extent of infiltration <10%; no FC done) and in another only by cytology (extent of infiltration 50–70%).

In one of two samples of angioimmunoblastic T-cell lymphoma, BM involvement was diagnosed only by histology and IHC.

Acute leukaemia

32 samples of acute leukaemia were studied (26 first diagnoses and 6 remission controls). For all acute leukaemias, both methods revealed concordant diagnoses in 27 of 32 samples (84%), i.e. in 21 of 26 AML samples (81%) and in all 6 ALL samples (100%).

In three of five discordant AML cases, BM involvement was detected by cytology only (extent of infiltration 50–70% in two cases and <10% in the third relapse case). In another discordant case,

Table 3

Diagnoses of 133 studied cases as confirmed by clinical follow-up (SLL/CLL = small lymphocytic lymphoma/chronic lymphocytic leukaemia, AML = acute myelogenous leukaemia, ALL = acute lymphocytic leukaemia).

Diagnostic groups and subgroups	No of cases confirmed by clinical follow-up
Lymphoproliferative diseases	50
SLL/CLL	13
Lymphoplasmacytic lymphoma	4
Mantle cell lymphoma	2
Follicular lymphoma	8
Hairy cell leukaemia	5
Plasma cell myeloma	10
Diffuse large B-cell lymphoma	5
Precursor T lymphoblastic lymphoma	2
Angioimmunoblastic T-cell lymphoma	1
Acute leukaemia	32
AML	26
ALL	6
MPS	10
MDS	10
Non-malignant alterations and normal BM	29
Metastases of solid neoplasms	2

Table 4

Concordance of histology and cytology in cases of lymphoma and acute leukaemia (note that this table comprises only 17 of the overall 26 discordant cases). The respective immunophenotyping methods used are given in the last four columns. (SLL/CLL = small lymphocytic lymphoma/chronic lymphocytic leukaemia, LPL = lymphoplasmacytic lymphoma, MCL = mantle cell lymphoma, FL = follicular lymphoma, HCL = hairy cell leukaemia, PCM = plasma cell myeloma, DLBCL = diffuse large B-cell lymphoma, PTLL = precursor T lymphoblastic lymphoma, AILD = angioimmunoblastic T-cell lymphoma; AML = acute myelogenous leukaemia, ALL = acute lymphoblastic leukaemia, FC = flow cytometry, IHC = immunohistochemistry).

diagnostic subgroups		No of cases	%	FC+IHC	IHC only	FC only	no immuno-phenotyping
SLL/CLL	concordant	12/13	92	6	6	–	–
	discordant	1/13	8	–	1	–	–
LPL	concordant	4/4	100	2	2	–	–
MCL	concordant	2/2	100	2	-	–	–
FL	concordant	4/8	50	2	2	–	–
	discordant	4/8	50	2	1	–	1
HCL	concordant	4/5	80	3	1	–	–
	discordant	1/5	20	–	1	–	–
PCM	concordant	7/10	70	4	3	–	–
	discordant	3/10	30	1	2	–	–
DLBCL	concordant	3/5	60	2	1	–	–
	discordant	2/5	40	–	1	–	1
PTLL	concordant	1/1	100	1	–	–	–
AILD	concordant	1/2	50	1	–	–	–
	discordant	1/2	50	–	1	–	–
AML	concordant	21/26	81	16	4	1	–
	discordant	5/26	19	1	–	2	2
ALL	concordant	6/6	100	6	–		

cytology diagnosed an AML while histology favoured a T-ALL and finally in one case, histology was compatible with an MDS, while cytology favoured an AML.

MPS

Cytology and histology were concordant in nine of 10 samples (90%). In one discordant sample, reactive alterations were described by histology.

MDS

Both methods were concordant in six of 10 samples (60%). In one discordant sample, cytology determined an MDS while histology was compatible with MPS. In three samples, MDS was diagnosed by histology only, while cytology favoured Non-Hodgkin-Lymphoma (NHL) in two cases and aplastic anaemia in one sample, respectively.

Non-malignant alterations

Histology and cytology were concordant in 25 of 29 samples (86%). In one of four discordant samples, histology indicated a T-cell lymphoma and in a second sample, cytology was suggestive of a follicular lymphoma. Clinical follow-up revealed an HIV-infection in both cases. In two other samples, histology suggested an MDS while cytology

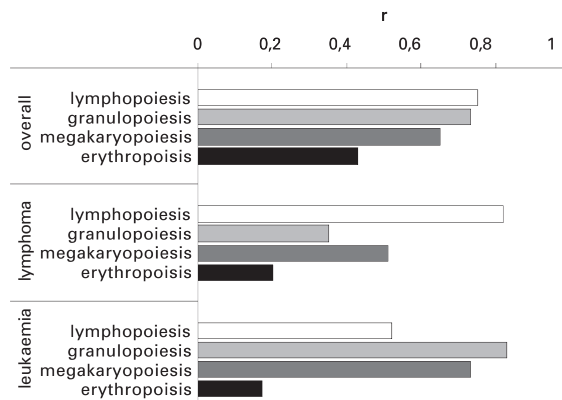

Figure 1

Correlation of cytology and histology for lymphopoiesis, granulopoiesis, megakaryopoiesis, and erythropoiesis in all samples, lymphoma samples and acute leukaemia samples.

was in favour of a deficiency in vitamin B_{12} and/or folate, which was confirmed by follow-up.

Metastases of solid malignancies

Both methods were concordant in two cases with metastatic BM involvement by a small cell lung cancer and a breast carcinoma, respectively.

Morphology

Cellularity and haematopoietic parameters

Comparison of cellularity, granulopoiesis, megakaryopoiesis, lymphopoiesis and erythropoiesis was feasible in 101 of 141 cases (72%: lymphoma n = 42, acute leukaemia n = 23, MPS n = 4, MDS n = 5, non-malignant alterations n = 25, metastases of solid malignancies n = 2). It is remarkable that cytological quantification of morphological parameters was not possible in six of 10 samples (60%) of MPS and in four of 10 samples (40%) of MDS due to inadequate smears. However, moderate to severe fibrosis was found in those MPS cases and an increase of reticular fibres in the respective MDS cases by histology.

Overall cellularity

Overall cellularity correlated well in general (r = 0.67, p <0.001), but varied between the different diagnostic categories. While correlation of cellularity was only moderate in lymphomas (r = 0.46, p <0.01, n = 42), it was good in non-malignant alterations (r = 0.61 p <0.01, n = 25) and excellent in acute leukaemias (r = 0.91, p <0.001, n = 23).

Haematopoietic data

Overall, haematopoietic data correlated well for lymphopoiesis (r = 0.75, p <0.001), granulopoiesis (r = 0.73, p <0.001) and megakaryopoiesis (r = 0.65, p <0.001). However, erythropoiesis displayed a considerably lower degree of correlation albeit still significant (r = 0.43, p <0.001).

In lymphomas (n = 42), correlation was excellent for lymphopoiesis (r = 0.82, p <0.001) but only weak to moderate for granulopoiesis (r = 0.35, p <0.05) and megakaryopoiesis (r = 0.51, p <0.001). Erythropoiesis showed no correlation at all (r = 0.20, p >0.05).

In acute leukaemias (n = 23), correlation was good for granulopoiesis (r = 0.83, p <0.001) and megakaryopoiesis (r = 0.73, p <0.001) but merely moderate for lymphopoiesis (r = 0.52, p <0.05). Again, erythropoiesis showed no correlation (r = 0.17, p >0.05). A comparison of overall correlation and correlation in lymphomas and acute leukaemias is shown in figure 1.

Cases of non-malignant alterations (n = 25) correlated moderately for lymphopoiesis (r = 0.49, p <0.05) and erythropoiesis (r = 0.50, p <0.05) but not for granulopoiesis (r = 0.27, p >0.05) and megakaryopoiesis (r = 0.33, p >0.05).

Blast counts

124 of 141 samples were assessable for blast counts. Both methods found an increased blast count in 40 of 124 cases (32.3%) and a normal count in 65 of 124 cases (52.4%). In general, concordance with respect to an increase in blasts was found in 105 of 124 cases (84.7%). In four of 124 cases (3.2%) histology found the blasts increased while cytology did not. In 15 of 124 cases (12.1%) cytology showed an increased blast count while histology did not.

BM infiltration by malignant disease

Assessment was possible by both methods in 133 of 141 cases (94.3%), excluding eight cases due to inadequate sample material. In 70 of 133 samples (52.6%), BM infiltration was found by both methods and in 45 of 133 samples (33.8%) by neither of the two. This results in a concordance in 115 of 133 samples (86.5%). In eight of 133 samples (6.0%), malignant infiltration was only detected by histology and IHC while in 10 of 133 cases (7.5%) it was detected by cytology and FC. However, one histological and three cytological putative tumour diagnoses were not confirmed by clinical follow-up data.

In summary, neoplasms (NHL, ALL, AML or solid malignancy), as confirmed by clinical follow-up, were found in 84 of all 141 samples. In 48 samples (57%), both FC and IHC were performed, in 26 samples (31%) only IHC and in four samples (5%) only FC was done. In six samples (7%), the neoplasm was defined on the basis of morphological findings only.

Histology (correct positive in 77 cases, false negative in 7 cases) and cytology (correct positive in 77 cases, false negative in 7 cases) had the same sensitivity (92%) for the detection of malignant disease but histology (false positive in one case, correct negative in 48 cases) showed a slightly higher specificity (98%) than cytology (false positive in 3 cases, correct negative in 46 cases; specificity 94%).

Comparison of degree of tumour infiltration in 56 samples (lymphoma n = 35, acute leukaemia n = 19, metastases of solid malignancies n = 2) revealed a good overall correlation (r = 0.64, p <0.001).

Table 5

Regression analysis between semi-quantified marker-expression as measured by IHC and FC.

	overall			lymphoma			acute leukaemia		
marker	r	p	n	r	p	n	r	p	n
CD45	0.93	<0.001	9						
CD20	0.87	<0.001	44	0.78	<0.001	23	0.93	<0.001	21
CD34	0.81	<0.001	28				0.87	<0.001	21
κ-light chains	0.71	<0.001	28	0.71	<0.001	21			
λ-light chains	0.62	<0.001	28	0.77	<0.001	21			
CD23	0.70	<0.001	19	0.56	<0.05	14			
MPOX	0.64	<0.001	25				0.49	<0.05	19
TdT	0.68	<0.01	14				0.67	<0.05	12
CD10	0.57	<0.05	12				0.57	>0.05	9
CD3	0.51	<0.001	38	0.25	>0.05	18	0.84	<0.001	20
CD5	0.46	>0.05	17	0.42	>0.05	11	0.54	>0.05	6
IgM	0.02	>0.05	13	0.07	>0.05	9			
CD7	0.07	>0.05	7				0.08	>0.05	6

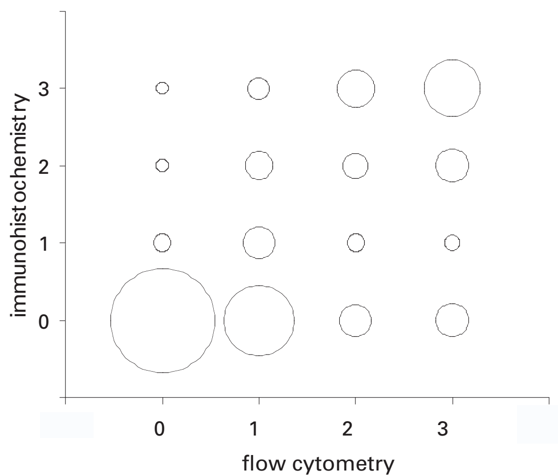

Figure 2

Epitope detection between IHC and FC according to semiquantified immunoreactivity (0 = <10%, 1 = 10–30%, 2 = 30–80%, 3 = >80%). All markers. Areas of circles represent the number of events.

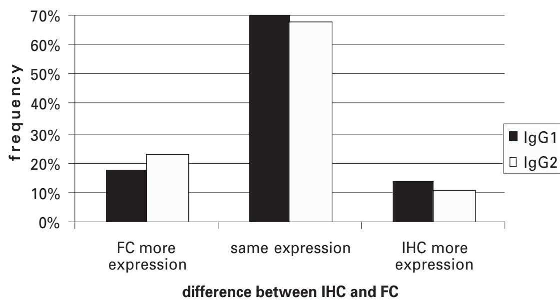

Figure 3

Differences of measured expression between IHC and FC dependent on different antibodies used for FC.

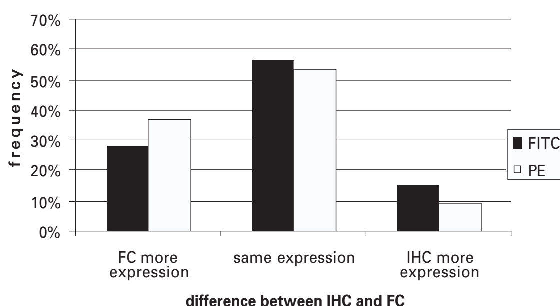

Figure 4

Differences of measured expression between IHC and FC dependent on different fluorochromes used for FC.

Immunophenotyping

The Wilcoxon test was used to examine whether there are fundamental discrepancies between immunophenotyping by FC and IHC. However, we found no significant differences for the immunoreactivity of several markers used (CD3, CD5, CD7, CD10, CD20, CD23, CD34, CD45, GLY A, MPOX, TdT, κ- and λ-light chains).

Regression analysis on the semi-quantitative expression of all immunological analysis performed by FC and IHC (n = 330) showed a linear relationship (r = 0.67, p <0.001, figure 2). However, differences in the degree of linear correlation between the individual markers were found as shown in table 5. Data for CD5, IgM and CD7 showed no correlation at all.

The respective data for the subgroups such as lymphomas and leukaemias are also shown in table 5.

Influence of IgG subclasses used for FC

We compared the differences between results of FC and IHC in cases where flow cytometric analyses were performed with IgG_1 antibodies (CD45, CD20, CD23, CD3, CD4, CD8, MPOX, GLY-A, CD34) to those where IgG_2 antibodies (CD10, CD5, CD7) were used. There was a significant difference between these IgG subclasses (p <0.001). Marker-expression in FC detected with IgG_1 antibodies (r = 0.76, p <0.001, n = 200) correlated better with immunohistochemical staining than marker expression detected with IgG_2 antibodies (r = 0.48, p <0.01, n = 40). With IgG_2 antibodies, FC showed significantly more expression than immunohistochemical stainings (p <0.01, figure 3), which was not the case for IgG_1 antibodies (p >0.1).

Influence of fluorochromes used for FC

We also compared the difference of expression with regard to two fluorochromes used for FC (fluorescein isothiocyanate [FITC] and phycoerythrin [PE]). Although the absolute difference between antibodies conjugated with FITC (CD45, CD20, CD10, CD23, IgM, κ, CD3, CD7, MPOX, TdT) or PE (λ, CD4, CD5, CD8, GLY-A, CD34) was only minute, it was significant (p <0.01). Expression of markers detected with FITC-conjugated

antibodies ($r = 0.70$, $p < 0.001$, $n = 234$) correlated slightly better with semi-quantified immunohistochemical staining than expression of markers detected with PE-conjugated antibodies ($r = 0.62$, $p < 0.001$, $n = 115$). With PE-conjugated antibodies, FC showed significantly more expression than immunohistochemical staining ($p < 0.001$, figure 4), which was not the case for FITC conjugated antibodies ($p > 0.05$).

Discussion

In this study, we compare the impact of aspirate smears and trephine biopsies in routine BM diagnostics as performed in our hospital. In general, the two procedures were concordant in 80.5% of cases, similar to published data [2, 5–7]. In contrast to previous studies, immunophenotyping was performed in a majority of cases in our study. Schwonzen et al. [13] showed that immunophenotyping in addition to standard cytomorphology improves diagnostic sensitivity for BM involvement by NHL. Since we studied cases selected for discussion on a BM board, we are aware of a certain preselection of cases. However, we believe that a putative selection bias – if present at all – would have lead to an overrepresentation of discordant cases and thus to a lower rate of concordant diagnoses.

The findings in the subgroups are in line with the overall results. In lymphomas, cytology and histology, combined with immunophenotyping, seem to be equivalent as a primary diagnostic approach. In some cases, the methods are complementary and thus both needed, as also described by Sabharwal et al. [14]. Nevertheless, plasma cell myeloma, particularly in cases with low infiltration degrees, was more reliably diagnosed in the biopsy, which is in line with findings by Pileri et al. [15]. Furthermore, follicular lymphomas with a low degree of paratrabecular infiltration were found only by histology. However, FC was not performed in these cases. In all discordant AML cases, the cytological diagnosis was confirmed by clinical follow-up. Such discrepancy seems to be related to the fact that single cell populations are more exactly quantified by cytology, which is crucial to discriminate AML from MDS. The finding that MDS are more accurately diagnosed by histology than cytology is noteworthy and could be a consequence of the case selection.

Since in two thirds of cases the discordance was due to the detection of the disease by one method only, while in one third of cases it was due to the identification of two different pathologies, this study indicates that discordant results are due to the different sensitivities of the various tools rather than to different specificities. However, this seems not to be true for MDS, MPS and non-malignant alterations. Both, biopsy sections and aspirate smears, should always be studied in these settings.

For morphologic parameters, there was a good overall correlation between semi-quantitative results obtained by cytology and histology, consistent with findings from other investigations [4, 7]. However, in contrast to the results of Ozkaynak et al. [3], we found that BM cellularity was equally well estimated by cytology and histology. Good correlation of lymphopoiesis in cases of lymphoma and of cellularity, granulopoiesis and megakaryopoiesis in cases of acute leukaemia can be easily explained by the histogenetic origin of the tumour cells. Quantification of morphology by cytology was not possible in 60% of MPS-samples and 40% of MDS-samples with increased reticular fibres as diagnosed in the trephine biopsy. In cases with severe BM fibrosis, aspiration of representative cell populations is often impossible, giving a particular value to the trephine biopsy in these cases.

In this study, cytologic evaluation detected increased blast cell counts four times as often as histology. Hence, in cases where quantification of particular cell populations is essential, cytologic specimens need to be obtained.

Our data show that both, cytology and histology, have the same sensitivity to detect malignancy in the BM. Thus, our results are at variance with some previous studies [16, 17], which favour histology as the better method to detect neoplastic disease in BM. However, it has to be stressed again that in our study immunophenotyping was additionally performed in the majority of cases.

Immunophenotyping is an objective and reproducible diagnostic tool for primary diagnosis and therapy control of haematological malignancies. Our results show concordance of immunoreactivities between FC and IHC for all markers. Published results for MPOX [18], TdT [19], CD34 [18, 20, 21], CD5 [22, 23], CD10 [22, 24–26], CD20 [22], and CD23 [22] are in line with our observations. Moreover, regression analyses on all immunological data show a good correlation between FC and IHC with the exception of IgM, CD5 and CD7, where no significant linear relationship was found. The results for lymphoma and acute leukaemia samples are consistent with the overall results, with the exception of CD3 in lymphomas and CD10 in leukaemias. At variance with the investigation by Kanter-Lewensohn et al. [27], we found that CD34 expression correlated well between IHC and FC in acute leukaemias. The discrepancies for IgM, CD5, CD7, CD3 and CD10 have not been reported so far.

Our results strongly indicate that the degree of correlation is dependent on the IgG subclasses and fluorochromes used for FC. Flow cytometric analyses performed with IgG_2 antibodies or PE as fluorochrome showed significantly more expression than IHC, which is in line with findings in the literature [28].

Antibodies against CD5, CD7 and CD10 used in our study for FC were IgG_2 antibodies, which might explain the lower degree of correlation with IHC-data. Furthermore, in our experience, IHC yields more variable CD10 immunoreactivities than FC. IgM-antibodies used for IHC and FC and CD3 antibodies used for IHC are polyclonal. This could explain the lower degree of correlation for these markers. However, in cases of acute leukaemia, CD3 expression is highly correlated between IHC and FC but only weakly in cases of lymphoma. These results may be explained by the fact that 75% of immunophenotyped leukaemias were CD3 negative.

Overall, our data indicate that the results for immunostaining by IHC and FC have a good comparability with regard to both quality and quantity of expression profiles.

In conclusion, our study shows that histology and cytology are of equal value in BM diagnostics in the vast majority of cases. The methods are often complementary and thus the respective materials (aspirate and biopsy) both need to be provided. In our experience, in some cases one or other of these methods is more conclusive. Cytology combined with FC usually allows exact quantification even of small, but immunophenotypically distinct cell subpopulations. On the other hand, histology and IHC allow in situ evaluation, which is particularly helpful with paratrabecular, low degree infiltration and/or cases with an increase in reticular fibres.

The constant pressure of public money shortage in health policy might fuel the assumption that the practice of the traditional twofold testing in bone marrow diagnostics is – in the light of the high rate of concordance of >80% – no longer necessary. However, the discontinuation of the one or the other examination might easily result in a loss of data and diagnostic accuracy. A more valuable time, money and resource saving alternative could be realized if a common primary approach in a comprehensive centre for bone marrow diagnostics were to be established.

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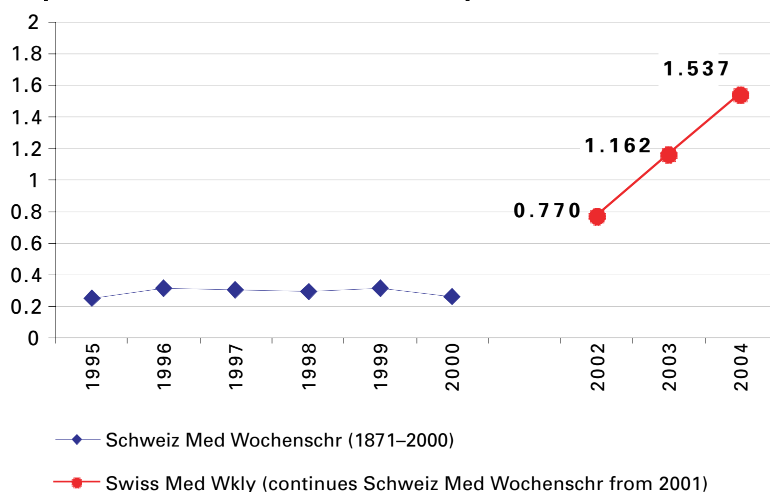

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