ORIGINAL ARTICLE

Clinical significance of plasmacytosis in the day+14 bone marrow of patients with acute myeloid leukaemia undergoing induction chemotherapy

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J Clin Pathol 2007;60:520–523. doi: 10.1136/jcp.2005.032870

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Accepted 18 March 2006
Published Online First
26 May 2006

Background: The design of chemotherapy-induction regimens for acute myeloid leukaemia (AML) is directed towards the early elimination of bone marrow (BM) leukaemic blast cells (LBCs). Patients with AML after induction show LBC reduction in a hypoplastic BM and also demonstrate a varying number of residual BM plasma cells (PCs).

Aim: To relate PC number to several blood and BM parameters as well as clinical features such as infection and survival.

Methods: On the 14th day after the start of chemotherapy (D+14) BM samples were examined for residual PCs in 60 adult (≥ 15 years) patients undergoing AML-induction chemotherapy, and the proportion of PCs was related to blood and BM parameters including French–American–British (FAB) subtype, other inflammatory cells, antecedent infection, attainment of complete remission and 36-month survival.

Results: Median PC proportion of 11.3% (range 0.1–48.7%) in D+14 BM aspirates and 10.7% (0.6–41%) in trephine biopsies was observed. Their number showed a direct relationship with residual BM lymphocytes ($r=0.368$; $p=0.025$). Higher numbers of residual PCs also reflected the presence of infection before diagnosis and coincident with treatment ($p=0.039$). Although we could not demonstrate an association between PC numbers and 36-month survival, PC numbers were significantly higher in patients with residual leukaemia at D+14 ($p=0.007$).

Conclusion: D+14 BM PC number reflects the effectiveness of induction chemotherapy and the presence of antecedent inflammation or infection.

Given that the cure of acute myeloid leukaemia (AML) mandates the attainment of complete remission, the first objective in treatment is the achievement of significant reduction in leukaemic blast cells (LBCs) early during chemotherapy. Thus, the bone marrow (BM) of such patients is usually examined at the 7th or 14th day (D+14) after the start of chemotherapy for evidence of the response.^{1–2} The D+14 post-chemotherapy BM is typically hypoplastic with rare haematopoietic cells, stromal cells, lymphocytes and plasma cells (PCs). The BM of some patients at D+14 can, however, show a marked increase in PC numbers, which can be found in aggregates and sheets that are sometimes so large that they simulate multiple myeloma. As far as we are aware, there are no published data concerning the number, clonality or significance of residual or increased PCs in the BM of patients in this setting, although there are reports describing the presence of plasmacytosis associated with acute leukaemia at presentation and before chemotherapy.^{3–4} We postulate that PC proliferation may exert an influence on the outcome of anti-leukaemia therapy.

In the present study, we examined if there was a relationship between: (1) the increased number of PCs and infections that occur commonly before or during induction therapy; (2) occult or overt antecedent chronic inflammatory conditions existing before initiating therapy; (3) residual PC numbers and the presence or absence of residual leukaemia; (4) the number of residual PCs and AML French–American–British (FAB) subtype; and (5) the clonality of residual PCs. Finally, we explored whether residual PCs had any effect on survival.

METHODS

We reviewed the case records of all adult patients with AML ≥ 15 years, diagnosed and treated between 1996 and 1998. The

review included the examination of archived D+14–17 post-chemotherapy BM slides for the presence or absence of residual leukaemia, lymphocyte aggregates, estimate of cellularity, and number and proportion of PCs per $\times 40$ high-power field in histological sections. In addition, differential counts for PCs, lymphocytes and histiocytes were performed on smears. We also performed immunohistochemical analysis for κ and λ light chains on all D+14 BM samples. The FAB classification of leukaemia at diagnosis was recorded together with the presenting blood haemoglobin (Hb), white cell and platelet counts. The clinical records were reviewed for history of fever and infection before the start of therapy (Day+0), and from Day+0 to Day+21 post-induction chemotherapy, and for remission attainment and survival data, as well as evidence of concurrent chronic inflammatory disorders, such as collagen vascular diseases.

Statistical considerations and analysis of data

Descriptive statistical analyses were undertaken for the retrieved data including the presenting peripheral blood Hb, white cells and platelet counts, blast cells and FAB subgroup, as well as for the data of the various BM parameters assessed on D+14 BM samples. These included the number and proportions of PCs per high-power field in histological sections and then of histiocytes and lymphocytes in the D+14 BM smears.

The presence or absence of infections preceding, coincident with or following (up to Day+21) induction chemotherapy was

Abbreviations: AML, acute myeloid leukaemia; BM, bone marrow; D+14, 14th day after the start of chemotherapy; FAB, French–American–British; Hb, haemoglobin; IL6, interleukin 6; LBC, leukaemic blast cell; PC, plasma cell

Table 1 Pretreatment French–American–British distribution of 60 patients with acute myeloid leukaemia

	M0	M1	M2	M3	M4	M5	M6	M7	MF	MDS-T
n (%)	(0) (0)	(4) (6.7)	(15) (25.0)	(7) (11.7)	(18*) (30.0)	(12) (20)	(0) (0)	(2) (3.3)	(1) (1.7)	(1) (1.7)

*Includes two cases of M4 eosinophilia.

Table 2 Morphological findings in D+14 bone marrow of patients undergoing induction chemotherapy

	≤10	<10–20%	>20%	>10–30	>30	No	Yes	Total
Cellularity		45	15					60
Residual leukaemia						52	8	60
Plasma cells (trephine/clot) (%)	26			26	7			59
Lymphocytes (%)	4			31	9			44
Lymphoid aggregates						50	7	57
κ/λ	60/60							

This table provides a general profile of the day >14 bone marrow features described in the text, and includes cellularity—for example, 45 of the bone marrow specimens had a cellularity of between <10% and 20%, and 15 had a cellularity >20%.

tabulated against the cellular parameters derived due to the BM studies.

The presence or absence of LBCs was also tabulated against the other cellular parameters. Using the Kaplan–Meier product-limit method, we estimated overall survival, defined as the interval in months between date of diagnosis and date of death from any cause. Log rank test compared overall survival between groups. A *p* value of <0.05 was considered significant for all statistical evaluations.

This study was approved by the clinical research and ethics committees of the King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

RESULTS

Between 1 January 1996 and 31 December 1998, we identified 60 patients, of whom 24 were men. The age ranged from 15 to 60 years, with a median of 29 years.

FAB grouping

Most FAB groups were represented, M4 being the most common, at 30%. No cases of M0 or M6 were seen (table 1). The majority of BM had been sampled at D+14 post-chemotherapy. Table 2 summarises the findings. No case of increased PCs, was observed in BM, either at diagnosis or before treatment.

Residual leukaemia at D+14

Morphological evidence of residual leukaemia was demonstrated in eight (13.6%) of the 60 patients at D+14 (table 2).

BM plasmacytosis

D+14 BM PC counts were determined in both aspirate smears and histological samples. In the former, the median (range) proportion of PCs was 11.3% (0.1–48.7%), and in the latter the respective value was 10.7% (0.6–41%; table 3, fig 1). When PC numbers in marrow smears were compared with those in trephine samples, there was excellent correlation, with a correlation coefficient of 0.890. PCs were present either as single cells, in small clusters or sheets. The latter profile was most evident in histological sections (fig 2). All 60 BM samples showed a polyclonal pattern by immunohistochemical analysis for κ and λ light chains.

In addition to PCs, we determined the proportions of other inflammatory cell types. Table 3 outlines the results.

Inter-relationships between PCs and other inflammatory cells

We next examined the inter-relationships of plasmacytosis with BM lymphocytes, lymphoid aggregates and histiocytes. Using BM PCs as the independent variable, we show a significant correlation between PCs and lymphocytes (*r* = 0.368; *p* = 0.025, two-tailed *t* test), but not with BM aspirate histiocytes (*p* = 0.257).

Plasmacytosis and infections in AML

As we have hypothesised that D+14 BM plasmacytosis could be a reflection of infection antecedent to, or after AML therapy, we identified patients who had infection prior to and co-incident with induction therapy and those who had no infection. Of the 59 patients whose charts were informative, 44 (74.6%) had no clinical history of infection before diagnosis, whereas 15 (25.4%) had an infection either before or coincident with treatment (table 4). The mean PC concentration in BM aspirates was 18.1% in patients who had infections compared with a PC concentration of 10.7% in those who were not infected. This difference is significant (*p* = 0.039, two-tailed *t* test).

Plasmacytosis and residual leukaemia

When D+14 bone marrow samples were examined for residual leukaemia, we found that the mean PC concentration in trephine biopsies in eight patients with residual leukaemia was

Figure 1 Frequency distribution of plasma cell (PC) numbers (proportions) in D+14 bone marrow (BM). Most BM samples have PC proportions between 7% and 28%. Counts were made on BM aspirate smears.

Figure 2 Patterns of residual plasma cells in D+14 bone marrow, comprising (A) scattered single cells, (B) cell clusters (C) and sheets. (A,B) are from Wright-Geimsa-stained bone marrow aspirate smears (original magnification $\times 400$) and (C) from H&E-stained trephine biopsy (original magnification $\times 200$).

significantly lower (4.6%), than in the 52 patients without residual leukaemia (14.3%; $p = 0.007$)

Survival

Only 19 (31.7%) of 60 patients were alive 36 months after diagnosis. Several variables including age, gender, presenting peripheral white blood, Hb, cells and platelet counts, BM histiocytes, lymphocytes, (aspirate or trephine), interval to diagnosis, post-induction infections, FAB subtype and presence

Table 3 General characteristics of D+14 bone marrow

	Mean (%)	Median (%)	Range (%)
BM histiocytes	8.28	6.0	0.1–57.2
BM lymphocytes	23.4	24.7	2.7–41.9
BM plasma cells smears	12.6	11.3	0.1–48.7
BM plasma cells trephine	12.9	10.7	6.0–41.1

BM, bone marrow.

This table summarises the quantitative parameters of patients, including their presenting peripheral blood counts, residual D+14 bone marrow inflammatory cells, and intervals between diagnosis and commencement of the therapy.

of residual leukaemia at D+14 were examined for the impact on survival. Kaplan–Meier analyses revealed no significant impact on median survival of any of these factors.

DISCUSSION

We demonstrate that residual PCs are present in the BM of some patients at D+14 post-chemotherapy for AML, with proportions varying from 0.1% to 48.7%. The PC proportions determined on BM aspirate smears were highly concordant with values derived from trephine samples ($r = 0.890$), supporting the robustness of our examinations.

We had postulated that proportion of PCs could be related to the presence of infection and/or inflammation before, or concurrent with, the onset and treatment of AML. Our review showed that of the 59 patients, 15 had an infection prior to or coincident with presentation and treatment of AML, whereas 44 others had no infection or other inflammatory process. Among the latter, the mean PC proportion (10.7%) was significantly lower than in the 15 patients who had infections (18.1%; $p = 0.039$). This relationship of

Table 4 Time points and types of Infection

Patient identification no	Organism	Day of positive culture
3	Ulcer, lower abdomen, coagulase-negative <i>Staphylococcus aureus</i> , tongue lesion	1–3
4	Blood, <i>Corynebacterium jeikeium</i>	6, 9
5	Mouth, HSV-1	17–21
14	Abdominal ulcer, <i>Pseudomonas</i>	7
17	Throat, group c β haemolytic <i>Streptococcus</i>	18
18	Tongue lesion, HSV-1	21
20	Right thigh abscess, <i>P aeruginosa</i>	21
21	Blood, coagulase-negative <i>S aureus</i> ; lip, HSV-1	14
25	Left axillary wound sinus, <i>S aureus</i>	14
27	FNAB right cervical area, <i>S. aureus</i>	18
36	Tongue, HSV-1	21
44	Central line inflammation	21
46	Central line, <i>Stenotrophomonas maltophilia</i>	20
56	Blood, coagulase-negative <i>S aureus</i> MRSA	5
59	Blood, <i>Pseudomonas aeruginosa</i>	14, 15
	Blood, <i>Enterococcus faecium</i>	15
	Blood, <i>P aeruginosa</i>	4
	Stool, <i>Dientamoeba fragilis</i>	18
	Blood, coagulase-negative <i>S aureus</i>	8
	IV tip, coagulase-negative <i>S aureus</i> and <i>Candida tropicalis</i>	4
	Ovary biopsy, coagulase negative <i>S aureus</i>	5
	Bronchoalveolar lavage, CMV	15

CMV, cytomegalovirus; FNAB, fine needle aspiration biopsy; HSV, herpes simplex virus; MRSA, methicillin-resistant *S aureus*

PCs with infection was underscored by the fact that the lymphocytes directly paralleled PCs with $p = 0.025$, $r = 0.368$. A similar relationship with BM histiocytes was not demonstrable, however, although it might have been expected that histiocytes, under the influence of increased interleukin 6 (IL6) secreted by LBCs⁵⁻⁷ would have been increased proportionately. Although no IL6 values were available in this study, the predominant FAB subtypes in this series had monocytic expression (30% M4 and 20% M5), and would have been expected to be associated with increased IL6 production⁷ and, consequently, increased BM histiocytes. As anticipated and borne out by the association of PC numbers with previous infection, all cases demonstrated polyclonal PCs.

We examined the question of whether D+14 BM PCs could predict the outcome in AML as has been observed in aplastic anaemia in which PCs could play either a positive⁸ or negative⁹ role in outcome. In this series of 60 patients with AML, it was clear that patients who had a greater proportion of PCs at D+14 were more likely to be free of leukaemia (PC concentration of 14.3% in 52 responders compared with 4.6% in eight non-responders). This observation does not translate into better medium-term survival, however, although a benefit may become apparent in a study involving a larger series of patients. Such benefit would be important to confirm as it would be consistent with the findings in some early studies that exposure to bacterial antigen, such as tuberculin, mediated a longer remission in acute leukaemia.^{10 11} The role of immunological mediation in maintaining remission after its induction in leukaemia has now been clearly demonstrated by stem-cell transplantation and other allogeneic cellular therapies, but a role for immunological therapies in remission induction is less clear. Our results, if confirmed, could provide the basis for further investigation in this area.

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Competing interests: None declared.

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