Virchows Archiv A Pathol Anat (1992) 420:473–478

Virchows Archiv A
Pathological Anatomy and Histopathology

The impact of megakaryocyte proliferation for the evolution of myelofibrosis

Histological follow-up study in 186 patients with chronic myeloid leukaemia

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Received October 14, 1991 / Received after revision December 24, 1991 / Accepted December 27, 1991

Summary. A histological study on sequential bone marrow biopsies in patients with chronic myeloid leukaemia (CML) was performed. We wished to answer the question as to whether a different content of megakaryopoiesis in the bone marrow of CML patients has a prognostic significance for the development of myelofibrosis during the course of disease. In addition, the significance of possible changes in the quantity of megakaryopoiesis in this process was assessed. In 186 patients who had no fibre increase at first diagnosis, the rate of subsequent myelofibrosis varied from 19% for the common or granulocytic subtype (CML.CT) to 40% for patients with features of megakaryocytic increase (CML.MI). No significant differences were found either in the rapidity of progression to fibrosis or in the final rate of osteomyelosclerosis. Whereas in CML.MI most patients (75%) showed an increase of fibres only, this was accompanied by an additional increase of megakaryocytes in CML.CT, changing the histological pattern from CML.CT to .MI or .MP, respectively. The data therefore revealed a correlation between fibre increase and subtyping of CML as suggested by the Hannover classification of chronic myeloproliferative diseases. Subtypes of CML with megakaryocytic increase could be shown to present a "pre-myelofibrotic" stage of disease and may therefore be conceived as a particular pathway of acceleration.

Key words: Histopathology – Bone marrow – Megakaryocytes – Myeloid leukaemia

Introduction

In patients with chronic myeloid leukaemia (CML), various grades of megakaryocytic proliferation can be observed in bone marrow biopsies or cytological smears at diagnosis (Georgii 1979; Burkhardt and Bartl 1982; Lorand-Metze et al. 1987; Lorand-Metze 1989; Thiele et al. 1990, 1991; Kaloutsi et al. 1991). These differences in the distribution of megakaryocytes are considered on the basis of our histological subtyping of CML, which discriminates four subtypes showing different grades of megakaryocytic proliferation (Georgii et al. 1990; Kaloutsi et al. 1991; Nafe et al. 1991).

Although there are some correlations with haematological findings, such as thrombocythaemic blood values (Buhr et al. 1987), the clinical significance, especially regarding life expectancy, is controversial (Buhr et al. 1987; Rozman et al. 1989; Thiele et al. 1988b, 1990, 1991). Therefore, a clarification of the biological relevance of the subtyping of CML requires further study.

Two different groups of CML, namely the "common" or granulocytic and the "megakaryocytic" subtype, were considered to be responsible for different outcomes in transformation of CML into myelofibrosis (MF) or progression to blast crises (Georgii 1979; Georgii et al. 1980). The prognostic relevance of a subtyping dependent on differential distribution of megakaryopoiesis may allow prediction of the course of histological progression with different risks of fibre increase, correlated to the number of megakaryocytes, as discussed previously (Georgii 1979; Georgii et al. 1980). This assumption was confirmed by other studies (Burkhardt and Bartl 1982; Burkhardt et al. 1984; Thiele et al. 1988a). However, in a recent study in this laboratory evaluating follow-up biopsies of CML patients, the former hypothesis of two strictly different entities in CML has had to be modified, since various transitions between both subtypes were found during the course of disease (Buhr et al., in press).

Since megakaryocytopoiesis is, in addition to other factors, intimately related to the development of MF (Castro-Malaspina and Moore 1982; Castro-Malaspina 1984), the question arises whether the different content of megakaryocytes is of any significance for progression to MF. This study was therefore performed to furnish evidence of possible differences in this respect between these subtypes of CML according to the Hannover clas-

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sification (Georgii et al. 1990), as well as to assess the meaning of possible transition within this process.

Materials and methods

All patients were recruited from the Bone Marrow Registry of the *Deutsche Krebshilfe* from a total of 55,728 biopsies referred for histological investigation between 1971 and 1987. Complete clinical data including date of initial diagnosis, cytogenetic status, treatment modalities, differential counts and clinical haematological values were evaluated from clinical files. Only patients with a typical clinical course of CML or positive Philadelphia chromosome, who had been treated by standard cytostatic regimens applying either busulphan or hydroxyurea, were included in this study. Two biopsies with a minimum interval of 6 months after initial examination were available. Thereby, a total of 229 patients with a total of 571 biopsies were included. Most of them (203) had up to three follow-up biopsies; 26 had four or more biopsies during their course of disease. The average follow-up time was 2.3 ± 1.8 years, ranging from 0.5 to over 10 years.

Bone marrow biopsies were performed according to the techniques described by Jamshidi and Swaim (1971) or Burkhardt (1966), respectively, and embedded in methyl-methacrylate after fixation in Hannover solution without decalcification. Further preparation included semi-thin sections (3 μm) as well as Giemsa, Gömöri, Masson-Goldner, and Prussian blue staining in each case (Vykoupil et al. 1976; Georgii et al. 1990).

Reclassification of all biopsies was performed according to the Hannover classification of chronic myeloproliferative disease (CMPD) (Georgii et al. 1990), which included the subtyping of CML according to the degree of proliferation of megakaryopoiesis into four subtypes (Fig. 1 A–D). Furthermore, a grading of fibrosis/sclerosis was performed, differentiating three grades of MF; the criteria are specified in Table 1. All biopsies which were inadequate due to technical procedures were excluded, leaving only representative specimens. This allowed a reliable sub-classification in each case.

Fibre increase was graded into three subtypes, based upon staining patterns and histological findings, as reported previously (Georgii et al. 1990; Buhr and Georgii 1991). Grade I was defined by slight increase of reticulin fibres in patchy, focal distribution recognized best by refringency in polarized light after Gömöri's silver staining. This pattern or grade is designated as early myelosclerosis (EMS); these borderline alterations cannot be recognized in decalcified bone marrow biopsies processed with usual staining. In grade II (MF), the fibre increase is more extended and intensive enough to be seen by trichrome staining methods and recognizable in biopsies processed with Gömöri's silver impregnation without polarization. Grade III, designated as advanced myelosclerosis or osteomyelosclerosis (AMF), is characterized by dense collagen fibrosis and marrow scarring, associated with intra-sinusoidal haematopoiesis and eventually by endophytic bone formation.

Table 1. Grading of fibre increase based upon the distinction of reticulin sclerosis (grade I), reticulin plus collagen fibrosis (grade II), and advanced fibrosis with diffuse, widespread extension

Grading	I EMS	II MF	III AMF
Fibres	Reticulin	Reticulin +collagen	Mostly collagen
Spread	Focal, patchy	Diffuse meshwork	Diffuse meshwork, scarring
Sinus		Sclerosis	Fibrosis, dilatation
Sinusoidal haematopoiesis	–	–/+	++
Endophytic bone formation	–	–/+	+ to +++

Results

Of the 229 patients, 192 were without fibre increase at initial examination (see Table 2). An exact grading of fibrosis and megakaryopoiesis was made in 186 cases which were considered in the following study.

The figures of progression towards MF/AMF are shown in Tables 3–5. There is a slight difference in the overall percentage of progression to fibrotic states, with a variance from 19% for common type CML (CML.CT)

Table 2. Reclassification of the initial or diagnostic biopsies from 229 patients with subtyping of the CML into four groups according to the Hannover classification: note the decreasing percentage of patients without fibrosis at initial examination within each subtype with increasing megakaryocytopoiesis

Grading classification	I EMS	II MF	III AMF	No fibre increase	Total
CML.CT	3	–	–	109	112
CML.OT	–	2	–	28	30
CML.MI	12	11	1	53	77
CML.MP	2	2	4	2	10
Total (*n*)	19	17	5	192	229

Table 3. Transition to different grades of myelofibrosis among CML.CT patients after initial examination; time to maximum grade of fibrosis is given for individual patients

Grading		Time (years)					
		<2	<4	<6	<8	≥10	Total
No fibres	*n*	50	22	7	4	2	85
I	*n*	3	5	–	–	–	8
II	*n*	4	4	3	–	–	11
III	*n*	–	–	1	–	–	1
Total	*n*	57	31	11	4	2	105

Table 4. Transition to different grades of myelofibrosis among CML.OT patients after initial examination; time to maximum grade of fibrosis is given for individual patients

Grading		Time (years)					
		<2	<4	<6	<8	≥10	Total
No fibres	*n*	8	8	3	2	–	21
I	*n*	3	2	–	–	–	5
II	*n*	1	–	–	–	–	1
III	*n*	–	1	–	–	–	1
Total	*n*	12	11	3	2	–	28

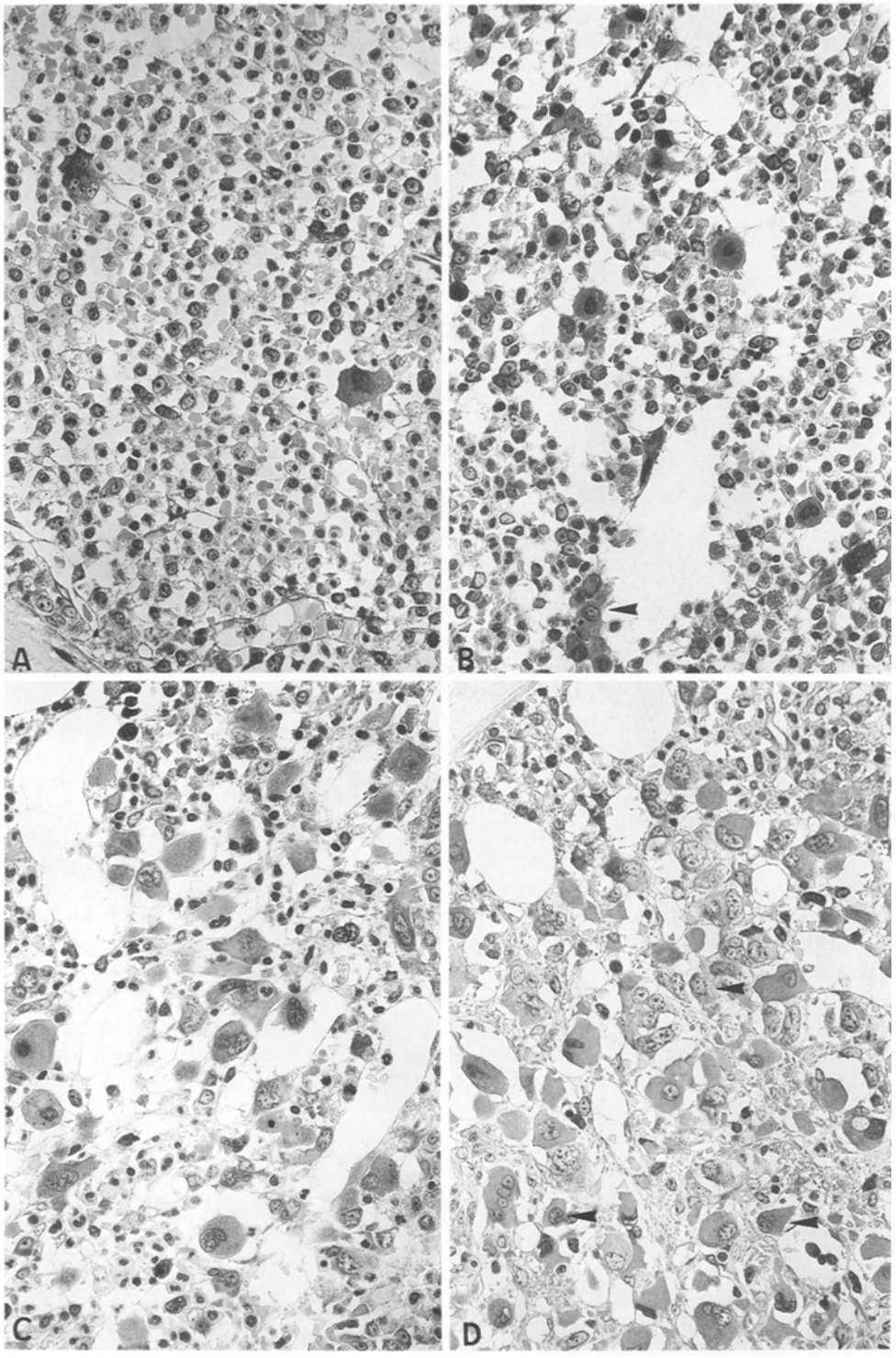

Fig. 1A–D. Subtyping of chronic myeloid leukaemia (CML) according to the Hannover classification of chronic myeloproliferative disease (CMPD) (Georgii et al. 1990). **A** Common (granulocytic) type (CML.CT) of CML, showing typical proliferation of granulopoiesis along with scattered megakaryocytes. **B** Overlapping type (CML.OT): apart from the proliferation of granulopoiesis, an increased number of megakaryocytes with occasional cluster formation (*arrowhead*), but irregular distribution within the bone marrow is found. **C** CML with megakaryocytic increase (CML.MI): overt uniform distribution of megakaryocytopoiesis nearly matching the rate of granulopoiesis can be seen, with frequent clustering of megakaryocytes. **D** Megakaryocytic predominance type of CML (CML.MP): a uniformly distributed, sheet-like arrangement of megakaryocytopoiesis is found, outnumbering granulopoiesis. Note the increased number of small precursors with roundish nuclei or "fried-egg"-shaped forms (*arrowheads*). Giemsa, ×25, 1:100

Table 5. Transition to different grades of myelofibrosis among CML.MI patients after initial examination; time to maximum grade of fibrosis is given for individual patients

Grading		Time (years)					
		<2	<4	<6	<8	≥10	Total
No fibres	*n*	20	10	1	–	–	31
I	*n*	10	5	1	–	1	17
II	*n*	–	–	–	–	–	0
III	*n*	1	2	–	–	–	3
Total	*n*	31	17	2	–	1	51

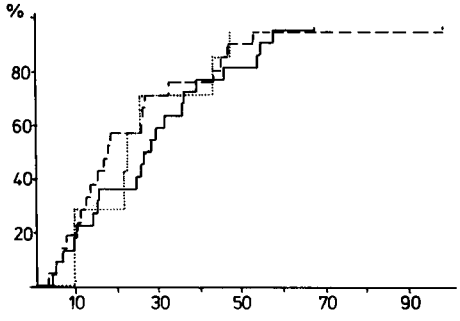

Fig. 2. Risk of development of fibrosis/sclerosis within subtypes of CML according to the Hannover classification. There are no significant differences for "median fibrosis time" (given in months on the *x*-axis) among the individual subtypes (cf. Table 6). (CML.CT = –; CML.M = – –; CML.OT = ...)

patients to about 40% for CML with megakaryocytic increase (CML.MI) graded forms. However, if the grading of fibrosis is taken into consideration, no significant differences could be found among the subtypes. Moreover, the percentage of patients with eventual severe MF (grade II, MF) was higher in CML.CT patients (Table 3), whereas most patients who had initially been subtyped among the CML.MI variant developed reticulin fibrosis. AMF was found in each group, but there was a predominance of the CML.MI group. Of the two initially non-fibrotic patients with CML with megakaryocytic predominance (CML.MP) one remained unchanged, whereas the other patient progressed to AMF. Figure 2 illustrates the rapidity of increasing fibrosis for each of the initial subtypes within the follow-up biopsies. The CML.MP patients were included in the group of CML.MI patients for this analysis. The median time until first appearance of any fibrosis was found to be shortest in CML.MI and longest in CML.CT (Table 6), but these differences were not significant on statistical analysis ($P > 0.05$).

Table 6. Median "fibrosis time" for CML patients without any fibrosis at initial examination until first appearance of fibrosis of any grade in sequential biopsies (cf. Fig. 2)

	Patients (*n*)	Median "fibrosis time" (months)
CML	192	24.5
CML.CT	109	26.0
CML.OT	28	23.0
CML.MI	55[a]	17.3

[a] Two CML.MP patients without fibrosis are included

The analysis of a scattergram of the time of maximum degree of fibrosis or megakaryocytopoiesis, respectively, within the follow-up of all CML patients without fibre increase at initial biopsy yielded four principal groups (Fig. 3).

The first group ($n = 138$ cases) consisted of those patients who displayed no increase in either variable. They are found at the cut-off point of the *x*- and *y*-axis, respectively.

The second group ($n = 22$ cases) was delimited comprising patients who had an increase of megakaryocytes only, corresponding to values on the *y*-axis.

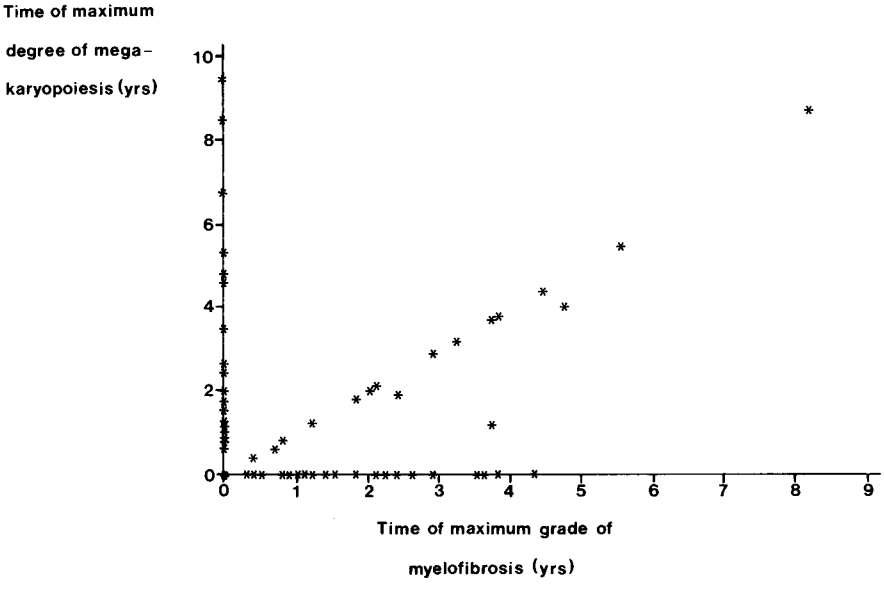

Fig. 3. Scattergram for times of maximum degree of fibrosis or megakaryopoiesis, respectively, within $n = 186$ CML patients, in whom no fibrosis at initial examination could be found (see text for further details)

Table 7. Distribution of CML patients with some degree of fibrosis in sequential biopsies according to subtypes of CML in initial biopsies. (cf. Tables 2–4 for further details of fibrosis)

Initial subtype	Fibres + megak. *n*	Fibres only *n*	Total *n*
CML.CT	13	7	20
CML.OT	3	4	7
CML.MI	5	15	20
CML.MP	1	–	1
Total	22	26	48

The third group of patients ($n=26$ cases) had some degree of fibre formation without change of the initial status of megakaryopoiesis (subtype of CML), whose values can be found on the x-axis.

A fourth group ($n=22$ cases) consisted of patients in whom a change of both megakaryopoiesis and fibre content was found in pertinent sequential biopsies. To our surprise, the maximum value of both variables was found in most patients within the same biopsies.

A total of 48 patients experienced some degree of fibre formation. Further analysis of these 48 patients reveals an interesting result (Table 7). In most patients who had initially been subtyped as CML.MI, an increase in fibres was not accompanied by further increase of megakaryocytes, whereas the opposite was shown in patients diagnosed as CML.CT. Patients with overlapping type CML (CML.OT) had an ambiguous course. Nearly half of them developed further increase of both fibres and megakaryocytes, whereas the other half developed an increase of fibres only.

Discussion

The only two reports hitherto published on sequential biopsies in CML suggested the hypothesis of a correlation between a high megakaryocytic cell count within the bone marrow and MF (Lazzarino et al. 1986; Thiele et al. 1988a). Thiele and co-workers have argued in favour of two different entities within CML, namely a "granulocytic" and a "megakaryocytic" form, revealing a different outcome with respect to blast crises or MF and corroborating a hypothesis formerly discussed elsewhere (Georgii 1979; Georgii et al. 1980; Burkhardt and Bartl 1982; Burkhardt et al. 1984; Frisch and Bartl 1985). However, this hypothesis has to be rejected when considering the data presented, since there were no essential differences in the eventual development of MF among all subtypes of CML. In addition, no significant difference in final stages of fibrosis and no basic differences in the rapidity of the development of fibre increase in the bone marrow could be detected among the various subtypes.

MF in CML is known to proceed to a final blast crisis (Gralnick et al. 1971; Devred and Diebold 1974; Buyssens and Bourgeois 1977; Clough et al. 1979) and is an unfavourable prognostic finding at diagnosis (Dekmezian et al. 1987; Thiele et al. 1988b, 1990, 1991). We therefore should consider whether MS and MF represent an acceleration of CML. Our data show a different propensity of the four subtypes of CML to progress to MF, since only 19% of CML.CT patients developed some degree of MF, whereas fibrosis ensued in nearly 40% of CML.MI patients. Although no significant differences in the time of development of fibroses could be detected in our material, it might have been possible to demonstrate their respective significance with an even greater number of patients, as "median fibrosis time" was different to some degree, and individual patient-related factors may have gained influence.

Moreover, a correlation was found between developing MF and an increase in megakaryocytes in CML.CT patients, whereas in CML.MI, no such correlation could be found. Therefore, it might be speculated that in most cases an increase in megakaryocytes will herald an unfavourable prognosis.

Returning to the question raised in the Introduction, concerning the significance and the impact of the Hannover classification of CML with its subgrouping of CML, there may indeed be some relation between MF and the subtyping of CML as proposed in the Hannover classification of CMPD (Georgii et al. 1990), since megakaryocytic subtypes may reflect a "pre-myelofibrotic" state of disease and may thus represent a particular pathway of acceleration apart from the well-known changes in granulopoiesis.

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