Review

Therapy-related changes of the bone marrow in chronic idiopathic myelofibrosis

J. Thiele[1], H.M. Kvasnicka[1], A. Schmitt-Gräff[2], R. Hülsemann[1] and V. Diehl[3]

[1]Institute of Pathology, University of Cologne, Cologne, Germany, [2]Institute of Pathology,
University of Freiburg, Freiburg, Germany and [3]First Clinic of Medicine, University of Cologne, Cologne, Germany

Summary. In chronic myeloproliferative disorders (CMPDs) a conflict of opinion exists regarding therapy-induced bone marrow (BM) changes and the evolution of myelofibrosis during the lengthy course of the disease. For a more elaborate study of these features chronic idiopathic myelofibrosis (IMF) seems to be a most suitable condition. Therefore this review is focused on this CMPD and amongst other findings analyzes data from a series of 340 patients with a long follow-up including 893 biopsies (median interval of 32 months). The ensuing results were compared with those communicated in the relevant literature. In addition to a control group of 153 patients with IMF who received only symptomatic treatment, therapy groups included busulfan, hydroxyurea, interferon and various combinations. In all groups hypoplasia of a varying degree was a frequent finding (6%) and often accompanied by a patchy arrangement of hematopoiesis. Most conspicuous was a gelatinous edema showing a tendency to develop a discrete reticulin fibrosis (scleredema). Aplasia developed in 7.7% of patients, usually at terminal stages of the disease independently of treatment. Minimal to moderate maturation defects of hematopoiesis involved especially megakaryocytes and erythroid precursors, but overt myelodysplastic features were most prominent following hydroxyurea and busulfan therapy. Acceleration and blastic crisis were characterized not only by increasing dysplastic changes, but also by the appearance of blasts including CD34^{+} cells. Semiquantitative grading of the fiber content revealed that 183 patients (54%) without or with moderate fibrosis at the beginning showed a significant progression and therefore contrasted with the 66 patients with a stable state. Following this calculation no relevant differences in the evolution of myelofibrosis were evident in the various therapy groups especially not following interferon treatment. In a few patients a regression was found which was accompanied by a severe hypoplasia or aplasia compatible with a myelo-ablative effect. In conclusion, peculiar BM changes, in particular conspicuously expressed myelodysplastic features are consistent with therapy-related lesions. Development of myelofibrosis in IMF is obviously due to disease progression unrelated to stage at diagnosis and not significantly influenced by treatment modalities.

Key words: Idiopathic myelofibrosis, BM changes, Myelodysplasia, Fibrosis, Chemotherapy, Interferon, BM biopsies

Introduction

Until now the impact of therapy upon clinical course and bone marrow (BM) morphology in Philadelphia-chromosome-negative chronic myeloproliferative disorders (CMPDs) has been very rarely and not systematically studied on larger series of patients. This may be due to the fact that most patients received significantly different and often cross-over regimens and during follow-up many clinicians were reluctant to perform additional BM biopsies (Barosi et al., 1990; Bilgrami and Greenberg, 1995; Gilbert, 1998; Bachleitner-Hofmann and Gisslinger, 1999; Tefferi, 2000, 2001; Spivak, 2002). On the other hand, as a sequel of chemotherapy a myelodysplastic and putative leukemogenic potential with a frequency of acute secondary leukemia ranging between 3% and 7% has been repeatedly described (Nand et al., 1996; Liozon et al., 1997; Rigolin et al., 1998; Sterkers et al., 1998; Finazzi and Barbui, 1999; Finazzi et al., 2000; Tefferi, 2001; Mavrogianni et al., 2002). Moreover, concerning dynamics of myelofibrosis in these disorders a number of conflicting studies have been published (Ellis et al., 1986; Hasselbalch and Lisse, 1991; Buhr et al., 1993; Georgii et al., 1998; Cervantes et al., 2002). Regarding these features amongst the different subtypes of CMPDs chronic idiopathic myelofibrosis (IMF) is considered an entity most suitable to study these therapy-related effects, because changes afflicting both compartments,

i.e. hematopoiesis and myeloid stroma (myelofibrosis), may be simultaneously investigated in this disorder.

Although in IMF some information is available concerning the pathomechanism (Le Bousse-Kerdiles and Martyre, 1999a,b) and especially the evolution of the fibrous marrow process (Thiele et al., 1988; Hasselbalch and Lisse, 1991; Buhr et al., 1993; Georgii et al., 1998) relatively little knowledge exists about therapy-induced changes (Buhr et al., 2003; Thiele et al., 2003). There has been general consent that conventional drug therapy in IMF is largely palliative and fails to improve survival (Barosi, 1999; Tefferi, 2000). In this context hydroxyurea (HU) is the most popular mode of treatment, although interferon α-2b (IFN) has been introduced as an alternate regimen, while busulfan (BU) is not used anymore because of its adverse reactions (Barosi et al., 1990; Gilbert, 1998; Bachleitner-Hofmann and Gisslinger, 1999). However, probably due to the already mentioned lack of repeatedly performed trephine biopsies during the lengthy course of IMF, characteristic BM features that may be caused by these drugs or which eventually occur as a natural sequel of disease have rarely been evaluated systematically in context with corresponding clinical data. Because response to therapy is very variable in these patients (Tefferi, 2000) it has been postulated that a synoptical approach considering simultaneously hematological and morphological data may further our understanding of the underlying disease process.

Consequently, this review is not only focused on results that may be gained from the pertinent literature (Thiele et al., 1989; Bartl et al., 1993; Buhr et al., 1993; Georgii et al., 1998), but also tries to include data derived from recently performed studies in this field (Buhr et al., 2003; Thiele et al., 2003). In this regard data from a retrospective evaluation of clinical records and BM biopsies derived from a series of 340 patients (156 males, 184 females; median age 64 years) that were recruited consecutively from 1982 to 1996, were also considered. According to the recently introduced WHO-criteria (Thiele et al., 2001) these patients presented with the full spectrum of IMF including prefibrotic-early (202 patients) to overt fibrotic and osteosclerotic stages (138 patients). Multiple sequential trephine examinations were carried out during the follow-up period with an observation time ranging from at least five to almost 14 years. Therapy was based on age, presence of poor performance status and complications like cytopenia,

organomegaly, hemorrhage and thromboembolic episodes and was adjusted to alleviate symptoms and avoid toxicity. Patients were separated into six different groups regarding their therapeutic modalities which are given in more detail in Table 1. The first group (control) received either no treatment or a continuous, occasionally also intermittent administration of antiplatelet drugs (i.e. aspirin and derivates) alone or in combination with other non-cytoreductive regimens including androgens and corticosteroids. Therapeutic modalities in the last group (group six) included multiple cross-over treatments or not other specified, intercurrent therapies between the biopsy intervals and finally, any treatment (in group two to five) for less than six months. A total of 893 representative BM trephine biopsies were performed: in 216 patients at least two, in 73 patients three, in 30 patients four, in 11 patients five, in seven patients six and in three patients up to 11 examinations. The initial trephine was always done at diagnosis with a mean interval of 46±62 months (median 32 months, range 6 - 759 months) between first and last biopsy. Major features of drug-related BM alterations are usually expressed by amount and distribution of hematopoiesis, maturation defects of the different cell lineages and changes in fiber content (van den Berg et al., 1990; Michelson et al., 1993; Wilkins et al., 1993; Rousselet et al., 1996; Hurwitz, 1997; Thiele et al., 2000) and may also be demonstrated in the majority of patients with IMF (Table 2).

Cellularity

Various degrees of hypocellularity, a patchy arrangement of residual or regenerating hematopoiesis, but especially a gelatinous (proteinaceous) edema with the tendency to generate a discrete network of reticulin fibers (so called scleredema) are amongst other changes believed to present characteristics of severe drug-induced or toxic myelopathy (Krech and Thiele, 1985; Wilkins et al., 1993; Hurwitz, 1997). These features are most prominent after myelo-ablative therapy (conditioning regimens) prior to stem cell or BM transplantation (van den Berg et al., 1990; Michelson et al., 1993; Rousselet et al., 1996; Hurwitz, 1997; Thiele et al., 2001). Other drug-associated BM features are more subtle and affect distinctive hematopoietic cell lineages like myelo- or granulopoiesis and the mononuclear-macrophage system (Thiele et al., 2000). On the other hand, concerning the assessment of

Table 1. Therapy in 340 patients with IMF.

TREATMENT	No. OF PATIENTS
1. none or supportive	153
2. Busulfan (BU)	30
3. Interferon a-2b (IFN)	26
4. Hydroxyurea (HU)	52
5. variable (mostly combinations of HU plus IFN and Ara C)	48
6. cross-over or less than six months	31

Table 2. Relative incidence (%) of prominent features of histopathology in our patients with IMF following therapy.

LESIONS	%
Hypoplasia	6.0
Aplasia	7.7
Maturation defects including myelodysplastic features	38.9

cellularity or extent of hypoplasia, one should be aware that there is a significant age-dependent influence on the amount of hematopoiesis, particularly in the superficial (subcortical) marrow spaces. Normally, in elderly patients these are occupied by adipose tissue, but in IMF an expansion of myeloproliferation can be detected in these areas. This phenomenon may be easily assessed in biopsy specimens performed in a proper orthograde direction and should be taken into account when grading cellularity. According to findings derived from our control group with only symptomatic treatment a gradual regression of hematopoietic tissue with replacement by fat cells, i.e. slight to moderate hypoplasia in addition to progressive myelofibrosis and adipocytosis, seems to be a spontaneously occurring event during the natural course of IMF. This cohort of patients without cytoreductive treatment showed an incidence in the sequential biopsy specimens of 5.3% especially at terminal stages, contrasting with the slight increase in the frequency of hypoplasia in the group treated with by HU (6.6%) and BU (9.1%). Opposed to this situation, pronounced hypoplasia and gross aplasia was found in 2% to 3% of the sequential trephine biopsies (Fig. 1a) independently from therapeutic modalities. In patients receiving no cytoreductive treatment this feature usually characterized BM changes at endstage disease (burnt-out stages) associated with hematopoietic insufficiency. Moreover, it has to be regarded that histotopography of the BM included a remarkable patchy arrangement of regenerating or residual hematopoiesis showing small clusters of early (macrocytic) erythroblasts and abnormal megakaryocytes with relatively large and dense nuclei (Fig. 1b). Interstitial changes were very conspicuous consistent with either a gelatinous (Fig. 1a) or fibrous edema characterized by a fine meshwork of reticulin, so-called scleredema (Figs. 1c, d). The stroma compartment also contained large numbers of iron-laden macrophages that were abundant after treatment and following transfusion therapy.

Myelofibrosis

Evolution of the myelofibrosis in IMF has been assumed to progress from an initial (hypercellular) stage without a relevant increase in reticulin to an advanced stage characterized by overt collagen fibrosis and optional osteosclerosis (Lohmann and Beckman, 1983; Thiele et al., 1989, 1999a, 2002, 2003; Georgii et al., 1990, 1998, 2002; Buhr et al., 2003). Recently, this concept has been validated by the new WHO-classification (Thiele et al., 2001) and some clinical data supporting the issue of early stage IMF presenting with no or little fibrosis in the BM (Cervantes et al., 1998; Tefferi, 2000; Buhr et al., 2003). Altogether laboratory features at the different endpoints of BM examination are supportive for a stepwise advancing disease process. Opposed to this finding disparate results have also been reported (Hasselbalch and Lisse, 1991) contesting a stage-like, but unpredictable progress of myelofibrosis in

the majority of patients (Wolf and Neiman, 1985; Barosi et al., 1988). In this context a conflict of opinion persits about a possible patchy distribution of fibrosis throughout the BM that may lead to non-representative biopsy specimens and significantly impair an exact quantification of the fiber content. According to autopsy studies where many sites could be examined some evidence has been produced that although no significant heterogeneity between various sites of examination is usually evident, minor differences in graduation may be encountered (Thiele et al., 1985; Kreft et al., 2000). These minor changes may explain our finding of a (one-grade) reduction in fiber density in six patients (Fig. 4) without previous cytoreductive therapy. Moreover, in a number of studies that reported adverse results concerning a progressively occurring myelofibrosis, patients entered either in advanced stage of myelofibrosis, where no relevant changes may be expected at follow-up or without a clear-cut diagnosis of IMF. Amongst others, these included terminal stages of polycythemia rubra vera presenting with postpolycythemic myeloid metaplasia (Wolf and Neiman, 1985; Barosi et al., 1988; Hasselbalch and Lisse, 1991) or even with the rather ill-defined entity of so-called acute (malignant) myelofibrosis (Hasselbalch, 1993).

Controversy and discussion arises when trying to quantify BM fiber content (Buesche et al., 2003). Although morphometry using the line intersection counting method with an ocular grid has been generally held to present a very reliable technique, it is a rather time-consuming approach to this problem (Thiele et al., 1999b, 2000). Therefore, for purposes of practicability, in grading of fibrosis (reticulin and collagen) a generally acknowledged semiquantitative scoring system should be preferred (Bauermeister, 1971) modified by corresponding morphometric data on the density of argyrophilic fibers (Thiele et al., 1996). Accordingly, the following scoring that has been widely used in relevant studies (Georgii et al., 1998; Buhr et al., 2003; Thiele et al., 2003) seems to be readily applicable: 0 - scarcely scattered linear reticulin with no intersections/cross-over, corresponding with normal BM; 1 - loose network of reticulin or focal increase especially around the vessel (sinus wall sclerosis) with many intersections (cross-over) in most areas of the section; 2 - diffuse and marked increase in reticulin with extensive intersections (dense network) and some bundles of collagen throughout the section; and 3 - diffuse and dense increase in reticulin and coarse bundles of collagen throughout the section, very often associated with osteosclerosis (endophytic bone formation).

It is noteworthy that at onset and during follow-up examinations a variable amount and quality of fibers was detectable in the repeatedly performed BM biopsies. These varieties ranged from an insignificant increase in fiber content (Fig. 2a) to early reticulin and gross collagen fibrosis, often associated with osteosclerotic changes of the bony trabeculae (Fig. 2c). When
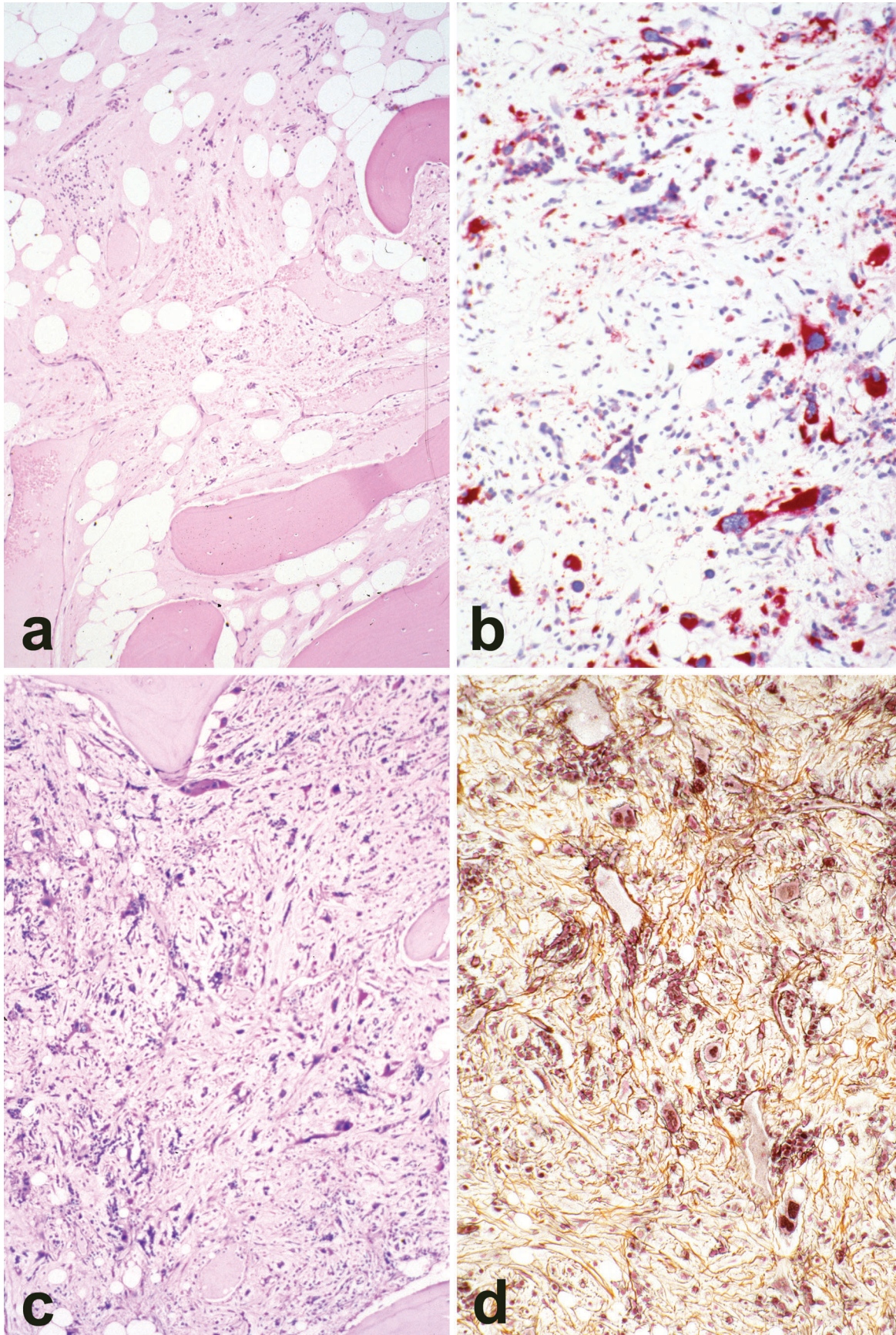
Fig. 1. Therapy-related bone marrow (BM) lesions in IMF. **a.** Following HU treatment aplasia with a gelatinous edema may evolve. **b.** A strikingly expressed patchy appearance of residual hematopoiesis is detectable after IFN therapy revealing small clusters of (macrocytic) erythroblasts and abnormal megakaryocytes with hypolobulated, dense nuclei. **c.** In some patients HU generates a dense sclerosing edema characterized by a fine network of reticulin, so-called scleredema **(d)**. (a) Hematoxylin/Eosin, (b) CD61 immunostaining, (c) PAS, (d) Silver impregnation; (a) x 80, (b-d) x 180
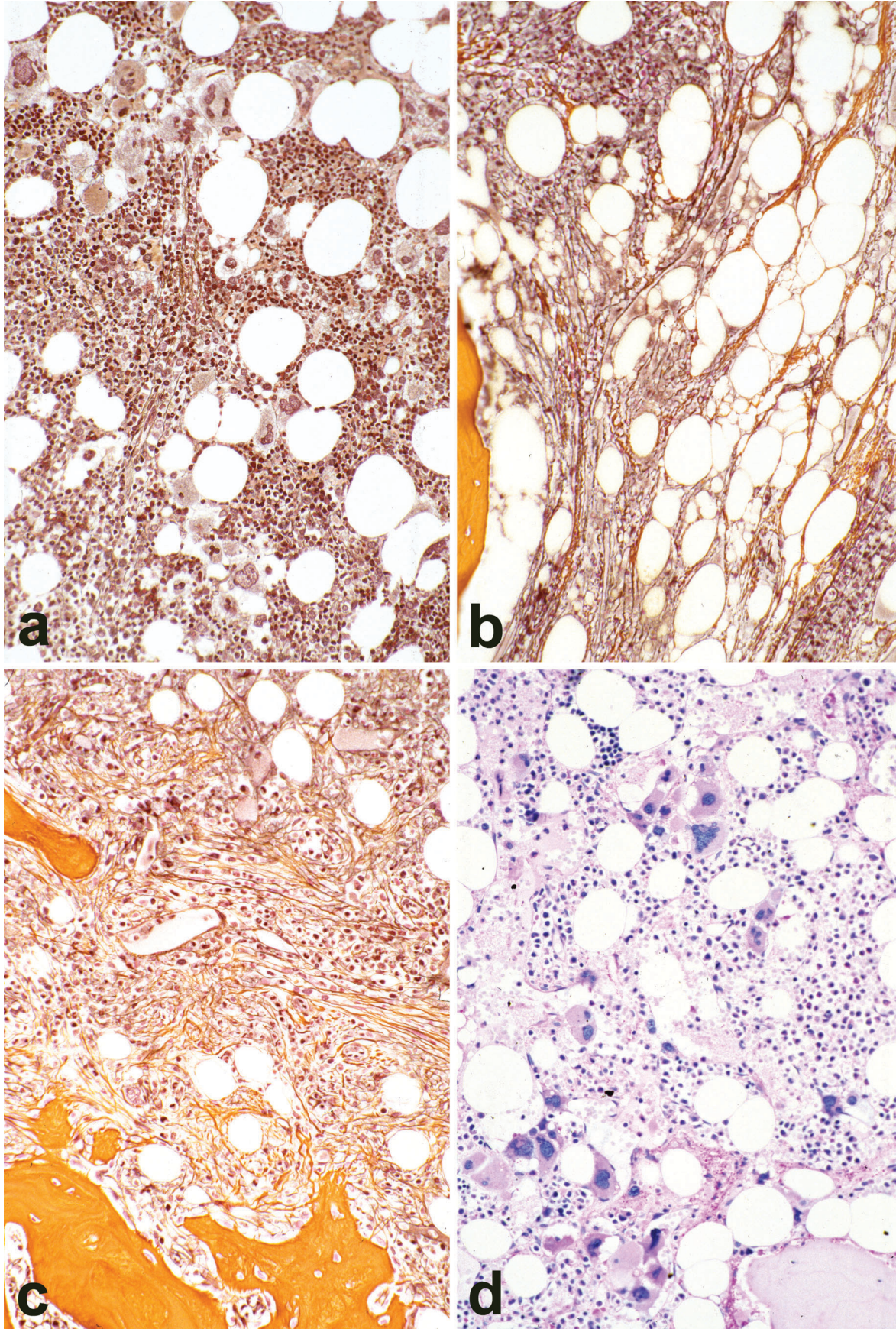
Fig. 2. Development of BM fibrosis in IMF. **a.** No increase in fibers (first biopsy on admission) consistent with a prefibrotic stage (IMF-0). **b.** Patchy distribution of hematopoiesis and a moderate increase in reticulin following IFN therapy for 12 months (IMF-1) in the same patient. **c.** Advanced fibro-osteosclerotic changes (IMF-3) arising from a prefibrotic lesion (first biopsy) in a patient with almost 10 years of symptomatic treatment (last examination). **d.** Prominent myelodysplastic features of mega-karyopoiesis following HU therapy for about 2.5 years include clustered small- to medium-sized megakaryocytes with hypolobulated (bulbous), dense nuclei surrounded by a relatively small portion of cytoplasm. (a-c) Silver impregnation, (d) PAS; (a-c) x 180, (d) x 380

considering the first and the last BM biopsy in each patient and the stage of IMF at diagnosis a general progression of myelofibrosis was evident in 183 of the 340 patients (54%) without or with only moderate fibrosis (grades 0 to 2) at onset (Fig. 3). This finding supports recently published data (Buhr et al., 2003; Thiele et al., 2003) that reveal a clear-cut tendency towards fiber increase in particular concerning patients with prefibrotic IMF (grade 0). Here a progression rate between 32% (Buhr et al., 2003) and 50 % (Thiele et al., 2003), depending on intervals between first and last BM biopsies (about 3 versus 4 years) was noted, thus implying a prodromal stage of overt (classical) IMF (Barosi, 1999; Tefferi, 2000). This feature was also explicitly expressed in the IFN-treated cohort of patients (Fig. 2a,b). A regression of fibrosis occurred in only 20 patients, while in 66 patients (excluding the 71 patients with the last grade of myelofibrosis or IMF-3, which remained constant by definition) a stable state was apparent. In this context it is noteworthy that the majority of patients with a regression of myelofibrosis which normally included only one grade, displayed severe hypoplasia to overt aplasia of the BM. This finding was consistent with a substantial myelo-ablative effect of treatment that was clinically associated with severe cytopenias and BM insufficiency (van den Berg et al., 1990; Michelson et al., 1993; Rousselet et al., 1996; Hurwitz, 1997). In six patients without cytoreductive therapy a grade-one regression of fiber density occurred during the course of the disease. In order to neutralize the shortcoming of variable biopsy intervals or endpoints of examination, cross-over of drug therapy and the significantly different numbers of trephines in each patient, we regarded the individual changes in the grades of fibrosis at a standardized median interval of 20 months (280 biopsy endpoints). When following this procedure calculation of possible changes in myelofibrosis revealed no significant differences in each therapy group implying a failing

influence of any treatment modality on the development of myelofibrosis (Fig. 4).

Although palliative therapy still remains the principal mode of treatment in IMF (Smith et al., 1988; Anger et al., 1990; Barosi, 1999; Tefferi, 2000), BM and stem cell transplantation have recently gained more acceptance, especially in younger patients (Anderson et al., 1997; Guardiola et al., 1999). Because IFN preferentially inhibits the proliferation of the megakaryocytic cell lineage and consequently interferes with the cytokine-mediated generation of myelofibrosis (Le Bousse-Kerdiles and Martyre, 1999a,b) this agent seemed to be particularly suitable for treatment (Gilbert, 1998). However, compared to HU as the drug of choice, IFN may not be well tolerated by many patients and, according to clinical data, was not shown to exert a clear-cut beneficial effect on the regression or inhibition of myelofibrosis (Parmeggiani et al., 1987; Barosi et al., 1990; Sacchi, 1995; Bachleitner-Hofmann and Gisslinger, 1999). As has been demonstrated by this study in most patients a progressively developing BM fibrosis that was not influenced by any treatment modalities, especially not by IFN, could be observed in IMF (Thiele et al., 2003). This result is in keeping with data from a recently published study on IMF including 109 patients of whom 48 received either BU or HU or combination therapies (Buhr et al., 2003).

Maturation defects

In addition to significant alterations concerning their quantity and distribution in the BM space (histotopography), hematopoietic cell lineages exhibited
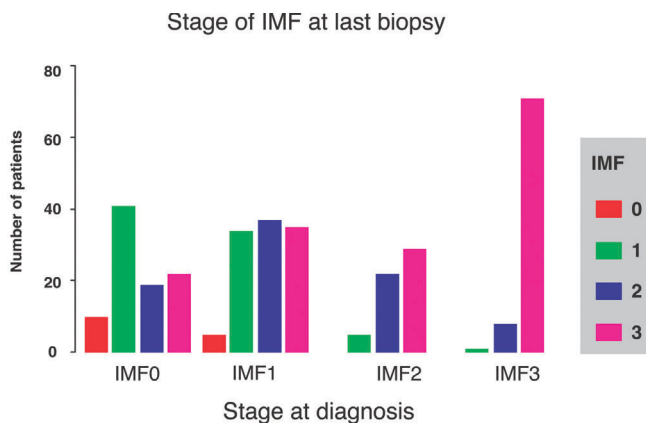

Fig. 3. Overview on the dynamics of myelofibrosis in our 340 patients with IMF regarding first and last trephine biopsies (median interval 32 months).
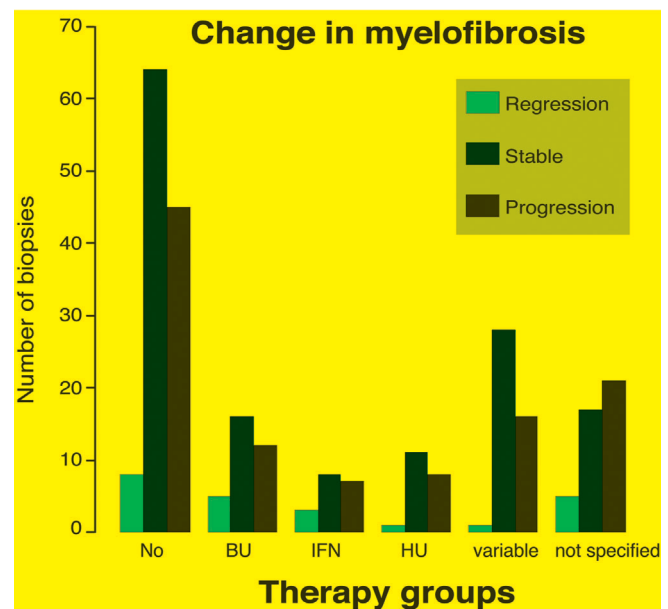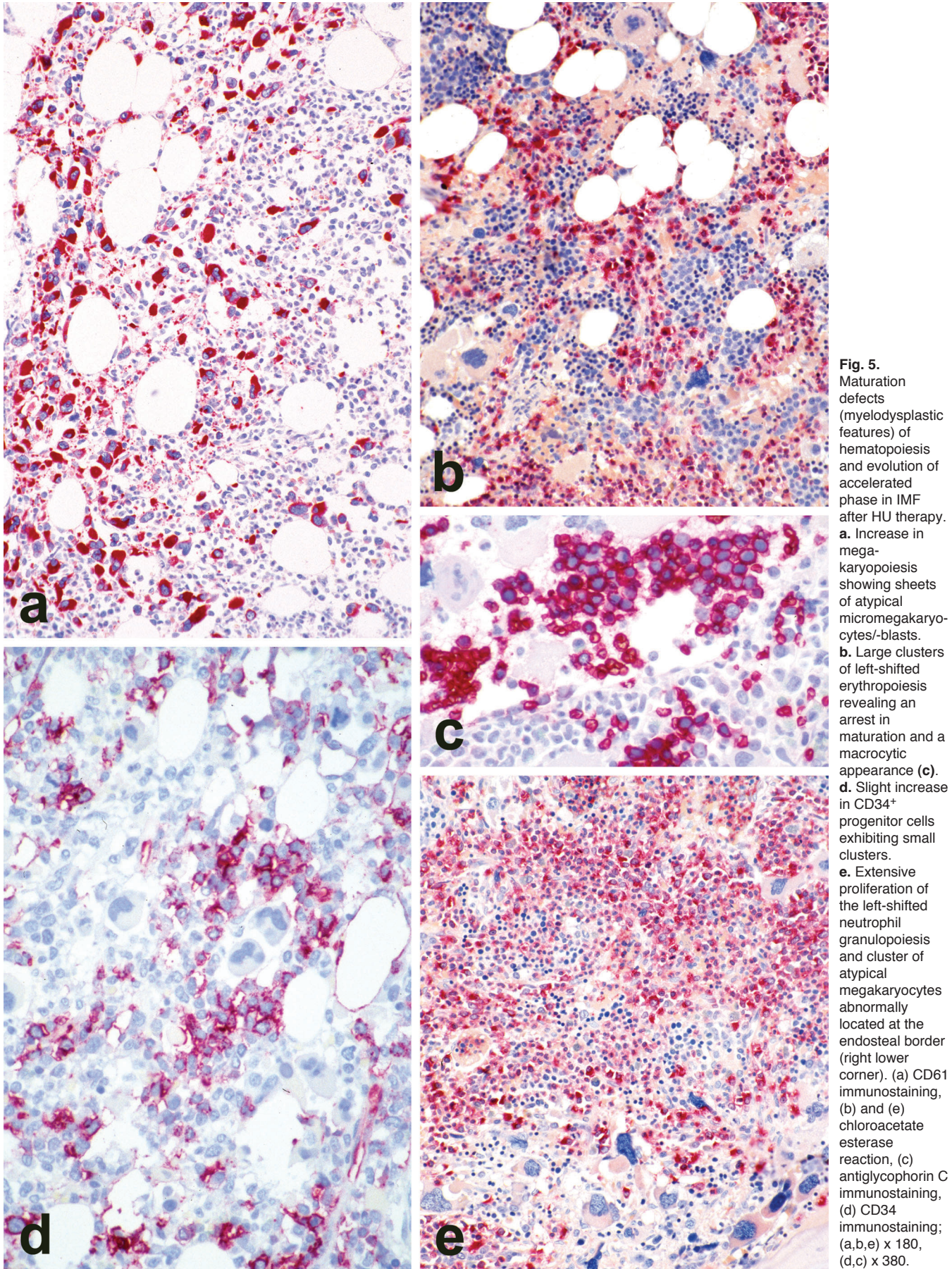

Fig. 4. Development of myelofibrosis in IMF according to 280 biopsy endpoints in the different therapy groups at standardized median intervals of 20 months (for abbreviations of therapy groups see Table 1).
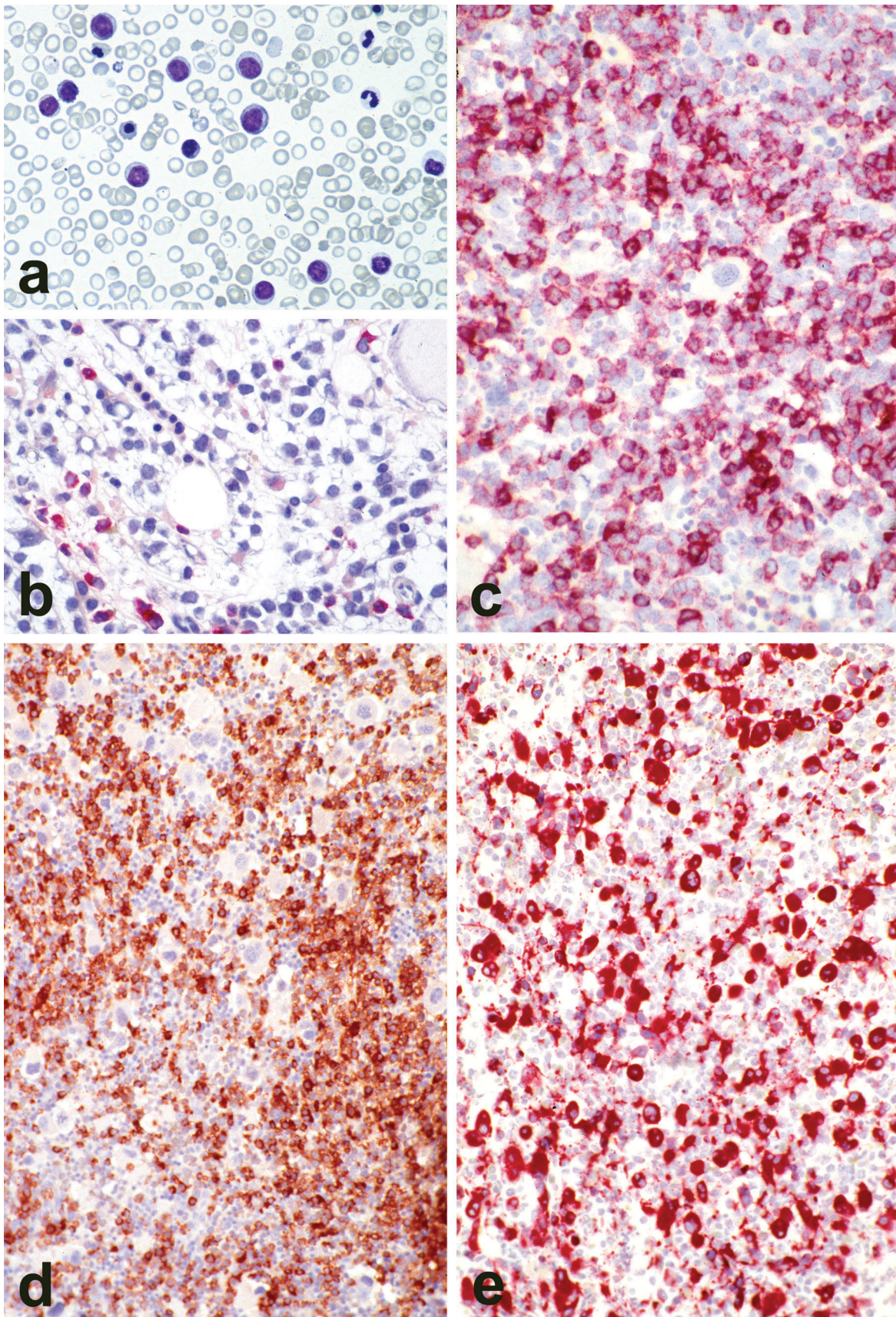
Fig. 5. Maturation defects (myelodysplastic features) of hematopoiesis and evolution of accelerated phase in IMF after HU therapy. **a.** Increase in mega-karyopoiesis showing sheets of atypical micromegakaryo-cytes/-blasts. **b.** Large clusters of left-shifted erythropoiesis revealing an arrest in maturation and a macrocytic appearance **(c)**. **d.** Slight increase in CD34^{+} progenitor cells exhibiting small clusters. **e.** Extensive proliferation of the left-shifted neutrophil granulopoiesis and cluster of atypical megakaryocytes abnormally located at the endosteal border (right lower corner). (a) CD61 immunostaining, (b) and (e) chloroacetate esterase reaction, (c) antiglycophorin C immunostaining, (d) CD34 immunostaining; (a,b,e) x 180, (d,c) x 380.

Fig. 6. Blastic crisis in IMF. **a.** Peripheral blood smear with pronounced leuko-erythroblastic reaction. **b.** Bone marrow showing a diffuse (chloroacetate esterase-negative) blast infiltration. **c.** Packed growth of CD34+ blasts. **d.** Extensive staining of (myelomonocytic) blasts with lysozyme expression. **e.** Overall proliferation of atypical micromegakaryo-cytes/-blasts. (a) Pappenheim, (b) chloroacetate esterase, (c) CD34 immunostaining, (d) lysozyme immunostaining, (e) CD61 immunostaining; (a) x 1080, (b,c) x 380, (d, e) x 180

various degrees of maturation defects at the different endpoints of examination during follow-up. At extreme ranges these changes were comparable to so-called secondary or therapy-related myelodysplasia which predominantly involved the erythroid and megakaryocytic cell lineages (Figs. 1b, 5a-c). In particular, megakaryocytes presented the diagnostic hallmark of these peculiar changes, since they showed a medium to small size and hypolobulated, dense nuclei surrounded by relatively small areas of cytoplasm and frequent clustering (Fig. 2d). Relevant changes ranged from occurrence of grossly atypical micromega-karyocytes (Fig. 5a) to a maturation arrest and macrocytic appearance of erythroid precursors (Figs. 3a, 5b,c), i.e. features that clinically were consistent with an acceleration. According to the repeatedly performed BM biopsies during evolution of the disease process borderline to mild maturation defects developed in about 23% of patients that received only symptomatic treatment, contrasting with the significantly expressed myelodysplastic changes in 3.3% patients of the HU group (Fig. 5a-e) that were not present after IFN treatment. It is reasonable to assume that minor to moderate maturation defects may develop during the normal course of disease even without interference by cytoreductive treatment. On the other hand, in 10 patients blastic transformation of IMF indicating terminal stages was characterized by an increase in the peripheral blast count (Fig. 6a) accompanied by overt immaturity of hematopoiesis (Fig. 6b). Differentiation of the blast population may vary considerably and often includes $CD34^+$ progenitors, lysozyme-expressing myelomonocytoid cells and $CD61^+$ (micro-) megakaryoblasts (Fig. 6c-e).

It should be emphasized that disturbances of maturation in CMPDs may present a diagnostic pitfall to hematopathologists, especially in those patients without any clinical information about previous therapy. Altogether conspicuous abnormalities of maturation may occur, which mimick myelodysplasia or so-called overlapping cases between a myeloproliferative and myelodysplastic disorder (Bain, 1999; Gupta et al., 1999). These difficulties are also highlighted in our series, because at the beginning we had to exclude 34 additional patients (14 females, 20 males) from further study. These peculiar cases showed IMF according to the first biopsy sample, but simultaneously exhibited strikingly expressed maturation defects of the erythroid and megakaryocytic lineage compatible with a myelodysplastic appearance. Although there was no indication of any preceding treatment on the report sheet, a more thoroughly performed investigation finally revealed a previous history of short-time cytostatic therapy. Since in this cohort no pretreatment biopsy specimens were available at diagnosis these patients were consequently excluded from this study. Therefore, much caution should be exerted, when diagnosing unclassifiable myelodysplastic/myelo-proliferative diseases in order not to miss any drug-related BM lesions.

Maturation defects ranging from mild anomalies of differentiation to overt myelodysplastic features have repeatedly been described (Frisch et al., 1986; Rigolin et al., 1998) in association with cytotoxic therapy in CMPDs (Sterkers et al., 1998; Randi et al., 2000). Significantly expressed myelodysplastic changes may precede leukemic transformation especially after HU administration (Loefvenberg et al., 1990; Weinfeld et al., 1994; Higuchi et al., 1995; Furgerson et al., 1996; Liozon et al., 1997; Finazzi and Barbui, 1999; Mavrogianni et al.,2002; Nielsen and Hasselbalch, 2003), thus contrasting with IFN therapy. In this context it is noteworthy that in our material myelodysplasia of megakaryocytes (so-called dysmegakaryopoiesis) was a most conspicuous feature following HU and BU administration and could be easily assessed (Figs. 2d, 5a, 6e), whereas changes of the other lineages were more discrete. However, one should not overlook the fact that maturation defects, mostly of a mild degree, were also found in the control group of patients that received only supportive treatment for many years. Therefore, defects of hematopoietic cell differentiation eventually evolve in the lengthy course of disease signalling terminal stages, independently from therapeutic modalities. It may be speculated that such changes indicate a severe disturbance of lineage-specific maturation (Fig. 5a-e) and may herald blastic transformation of the disease process in a number of patients (Fig. 6a-e).

Clinical features

In keeping with the consideration of early to advanced stages of IMF at diagnosis of patients (first biopsy) clinical data showed a striking heterogeneity which, however, differed from the findings following therapy at the time of the last biopsy (Smith et al., 1988; Thiele et al., 1989, 1996, 2002; Cervantes et al., 1998; Georgii et al., 1998; Buhr et al., 2003). The 202 patients presenting with initial to early IMF without or with mild reticulin fibrosis were characterized by a more frequently occurring thrombocythemia exceeding $1,000x10^9$/L (34%), a leukocytosis greater than $10x10^9$/L (52 %) and a minimal to mild anemia (38 %) or splenomegaly (15%). In more than 20% of the patients of this cohort there was a relevant rise in the LDH value and the score of the leukocyte alkaline phosphatase (ALP) detectable. In a minority (4 %) of these patients a borderline to slightly expressed leuko-erythroblastic blood picture with atypical precursors and tear-drop poikilocytosis appeared on the blood films. At the endpoint of the last BM examination during the lengthy course of the disease differences concerning laboratory features of this group were not clearly distinguishable from the 138 patients that were initially diagnosed as overt (classical) IMF (Barosi, 1999; Tefferi, 2000). The latter cohort already presented with myelofibrosis, anemia, splenomegaly and tear-drop poikilocytosis at the beginning. The straightforward

evolution of clinical data during follow-up underlines the dynamics of the disease process in IMF. However, when discriminating patients according to their therapy groups hematological variables failed to display different constellations of findings. In 10 patients a leukemic transformation was recognizable which occurred besides severe BM insufficiency as cause of death. In this context the myelodysplastic and putative leukemogenic effects following chemotherapy with HU in CMPDs have to be emphasized (Nand et al., 1996; Liozon et al., 1997; Rigolin et al., 1998; Sterkers et al., 1998; Finazzi and Barbui, 1999; Finazzi et al., 2000; Mavrogianni et al., 2002; Nielsen and Hasselbalch, 2003). This feature merits some attention because contrasting with IFN administration, prominent maturation defects were observed in a considerable number of our patients in particular following HU treatment.

In conclusion, this review has systematically outlined drug-related lesions of the BM in IMF and in particular has focused on changes in cellularity, histotopography of residual hematopoiesis and the occurrence of maturation defects, including myelodysplastic features. Follow-up in a large series of patients (Buhr et al., 2003; Thiele et al., 2003) has not only validated the recently introduced diagnostic criteria of the WHO (Thiele et al., 2001), but also emphasized that evolution of myelofibrosis is not substantially modified by therapy.

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