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Evolution Of Clonal Cytogenetic Abnormalities in Aplastic Anemia

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Prior to the introduction of effective therapies, the high mortality rates of severe aplastic anemia (AA) precluded recognition of late complications of this disease. Once the survival of AA improved, observation of clonal evolution raised questions as to whether the development of secondary myelodysplastic syndrome (MDS) is a part of the extended natural history of the disease or is related to the therapies applied. Clinical features of myelodysplasia and AA can overlap, and typical MDS may evolve as a complication of AA. Common pathophysiologic elements operate in these diseases and are subject to many studies and theories as to what mechanisms in AA may lead to the late evolution of MDS. Similarly, AA has been hypothesized to be a reflection of an over-reactive immune response triggered by the appearance of genetically altered and/or phenotypically abnormal dysplastic clones. Hypocellular variants of myelodysplasia and responsiveness of certain forms of MDS to immunosuppressive regimens serve as the most appealing examples of the intricate and close pathophysiologic relationship of this disease with AA. The diagnosis of clonal evolution in the course of AA can be obvious if secondary cytopenia involves hypercellularity and a high percentage of blasts. In addition, the occurrence of a new karyotypic defect objectively heralds the progression of disease to MDS. However, the diagnostic imprecision of dysplasia recognition in the context of marrow hypocellularity, inability to obtain informative cytogenetics, and a high proportion of MDS cases with normal karyoptype have hampered studies designed to determine the frequency and timing of MDS evolution in AA. In addition, the diagnostic criteria and definitions used are not unified. While some centers recognize that the abnormal karyotype does not preclude the diagnosis of AA; in others, the diagnosis of AA includes the presence of normal karyoptype. Many typical features of dysplastic evolution in AA have been clarified. For example, karyotypes most frequently encountered in MDS secondary to AA involve chromosomes 6, 7 and 8. The evolution rates seem to be in the range of 10–15% in 10 years, but there are no predictive clues as to which patients are at greatest risk for this complication. Study of the mechanisms of clonal evolution in AA may help understand the pathophysiology of other forms of MDS and leukemia and also the mechanisms of antileukemic surveillance. Clinically, identification of patients at increased risk for clonal complications may influence the choice of therapies applied.

Keywords: Aplastic anemia; Myelodysplasia; Karyotypic analysis; Clonal evolution

INTRODUCTION

With the introduction of immunosuppressive therapy for aplastic anemia (AA), the survival of patients who are not treated with bone marrow transplantation has improved significantly [1], and with long-term systematic observation, evolution of AA to other hematologic diseases has been frequently recognized as a serious late complication of this disease. Historically, the development of paroxysmal nocturnal hemoglobinuria (PNH) was considered the most common clonal complication of AA. Although the appearance of PNH clones is often already observed at first presentation of bone marrow failure [2,3], manifest PNH develops in a much smaller but significant proportion of patients. Myelodysplastic syndrome (MDS) is the second most common clonal disease occurring in the context of AA. Aberrant differentiation of hematopoietic precursor cells, increased numbers of myeloblasts, and marrow hypercellularity are all characteristic of MDS, but persistent bone marrow hypocel-

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lularity in AA may preclude reliable morphological analysis. Consequently, the finding of a cytogenetic defect appears as the most objective evidence of clonal evolution to MDS.

Theories of MDS Evolution in the Context of Pathophysiology of AA

A fundamental question in the evolution of clonal disease in AA is whether its pathophysiology is intrinsic to the natural history of AA, and now only observed as patients survive longer due to effective therapies. Alternatively, clonal evolution may be secondary to the treatment of AA, as a complication of immunosuppressive therapy, or more recently, due to chronic growth factor administration. Theoretically, inhibition of immune surveillance could lead to the uncontrolled outgrowth of abnormal clones. However, MDS has occurred in AA treated with androgens only [4,5] arguing against the theory that the clonal evolution to MDS is a consequence of immunosuppression. In addition, patients with primary MDS have been treated with immunosuppressive agents and no acceleration of the disease has been observed [6–8]. While prolonged G-CSF treatment was linked by Japanese investigators to the evolution of monosomy 7 [5,9–11], there was no increased risk observed in a randomized study of ATG and CsA with and without G-CSF [12] or in the analysis of EBMT® data [13]. In the NIH study [14], many of the AA patients who developed cytogenetic received hematopoietic growth factors, mainly as support or as salvage therapy after unsuccessful immunosuppression, and refractory disease itself may be the underlying risk factor for clonal evolution. Most AA patients who do not undergo bone marrow transplantation will be treated with immunosuppression and often hematopoietic growth factors. In the absence of appropriate control groups, it is difficult to rigorously establish or exclude a pathophysiologic relationship between clonal evolution and specific therapies [11].

Clinically, karyotypic abnormalities appear to concur with other features of typical AA such as the high incidence of HLA-DR2 [15] and coexistence of an expanded PNH clone [2]; patients who show cytogenetic evolution do not belong to a distinct nosologic subtype, as might be due to the misdiagnosis of MDS at the outset of their disease. While it is likely that hematopoietic stem cell clones are ultimately selected by their growth characteristics, the nature of their growth advantages may vary. Possible pathophysiologic mechanisms may include unregulated proliferation due to defects in cell cycling, or apoptosis in preleukemia, compared to aberrant responses to immune stimuli, as may be the case for trisomy 8 [16].

Several theories can be formulated with regards to the link between AA and MDS (Fig. 1) [17–19]. The late occurrence of abnormal clones as a complication of AA could be a result of the pathophysiology leading to AA. For example, karyotypic abnormality may be acquired and selected during an immune attack on hematopoietic progenitor cells. However, it is also possible that abnormal hematopoietic clones may be present prior to the development of AA and carry new antigens that can incite the autoimmune process leading to AA. Whether more sensitive methods such as fluorescent *in situ* hybridization would have disclosed small populations of aberrant cells at earlier time points remains unclear.

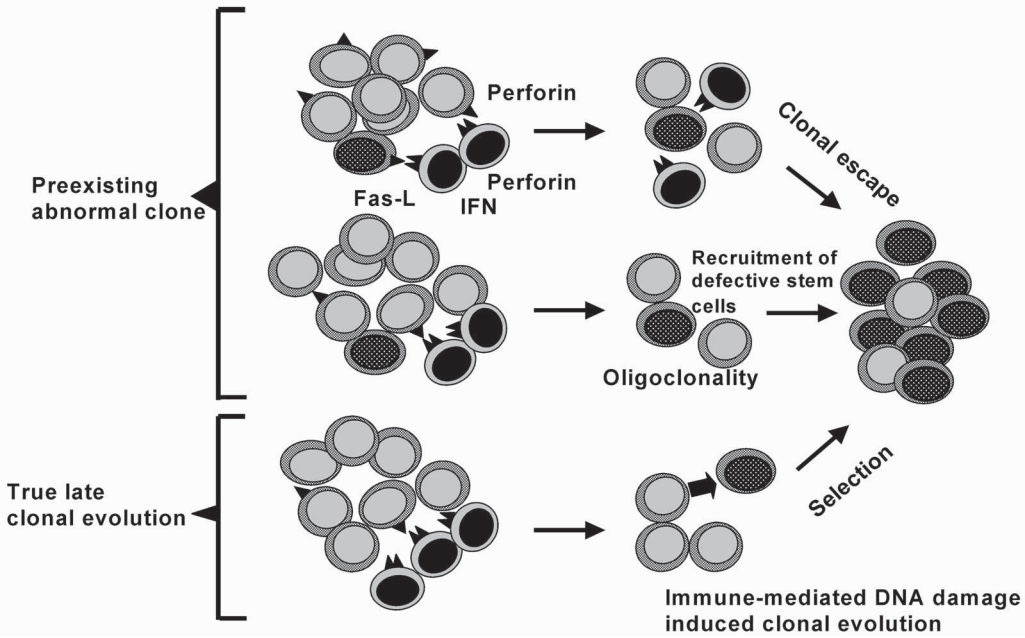

FIGURE 1 Theoretical mechanisms of clonal evolution in AA. Two upper pathways show possible mechanisms based on the theory that the abnormal stem cell clone predates development of AA. The possibility that immune-mediated attack on hematopoietic target may by itself lead to DNA damage is depicted in the lower portion of the graph.

However, were under-diagnosis, due to inadequate techniques for the detection of chromosomal changes, important, evolution to MDS would be more rapid (due both to emergence of a pre-existing clone and to the absence of immune surveillance resulting from therapy), but in most studies, chromosomal changes emerged gradually over time, a process, more consistent with stochastic kinetics than a determinative process.

In a contrasting hypothesis, development of MDS may be consistent with only a partial ability to mount an immune response to such antigens and inhibit clonal evolution (Fig. 1). Therefore, it is possible that a pathophysiologic continuum exists from frank aplasia, hypoplastic MDS (with an immune response consistent with autologous graft-vs.-leukemia effect) to refractory anemia with blast excess in which immune mechanisms are ineffective or selection pressure leads to clonal escape. However, immunosuppression in such a setting would have a promoting effect on the progression of MDS, a phenomenon that has not been observed in clinical trials [11,18].

Diagnosis of Evolution, its Frequency and Timing

The relationship between AA, a disease dominated by an immune pathophysiology similar to other organ specific autoimmune diseases, and MDS, usually viewed as a pre-malignant process, remains unclear in many clinical and pathophysiologic aspects.

The development of MDS in the setting of diagnosed AA has been described in several studies (Table I), but these vary significantly in the design and especially in case definition [10,20–26] exemplifying diverse views with regard to the criteria required for the diagnosis of both MDS and AA. In historical studies of AA, patients with abnormal cytogenetics and hypoplastic marrows at presentation were often included [27–30], and in some institutions, abnormal cytogenetic studies are compatible with a primary diagnosis of AA [4,30–32].

Most commonly, abnormal cytogenetics was felt to exclude a diagnosis of AA, regardless of marrow morphology; in a series from the NIH involving 122 patients treated with intensive immunosuppression consisting of anti-thymocyte globulin (ATG) and cyclosporine A (CsA), all patients showed a normal karyotype at presentation, and 14 have subsequently developed karyotypic abnormalities, with a risk of about 21% at 10 years. Only 2 patients were diagnosed as a late MDS by marrow morphology alone, with normal chromosomes [27]. In an early study from Seattle, an abnormal karyotype was reported in 7 out of 183 AA patients, but only 3 of these developed after immunosuppression [4]. The differences in the diagnostic criteria are also obvious such as in a recent analysis by the EBMT Aplastic Anemia Working Party, in which karyotypic abnormalities occurred in 23 out of 170 patients, but in 4 cases chromosomal changes were present at first diagnosis [27] and would be classified as MDS at other institutions. Similarly, in a recent British series of 13 patients with AA and abnormal cytogenetics, only 2 presented with normal karyotype and later developed an abnormality [31]. In a study of 159 children with AA from Japan, the authors identified 6 patients with the diagnosis of "AA with cytogenetic abnormalities" [30]. In another cohort complied of 100 patients from the GITMO and EBMT study involving ALG, CsA prednisone and G-CSF, during a median follow up of 1,424 days, 11 patients developed cytogenetic abnormalities [33]. In the interval of 11 years, 8% of patients enrolled in the randomized ATG + / − CsA study developed MDS or AML [34].

Another important problem in assessing progression of the disease to myelodysplasia is the inability to obtain informative chromosomal analysis at presentation, or lack of information on the karyotype at presentation. For example, in a report of 69 Italian AA patients, only half the group had cytogenetic testing performed at the time of diagnosis of AA; an abnormal karyotype was found in 18, but the findings were transient in 8 patients and in

TABLE I Selected studies on karyotypic abnormalities

	Patients with aplastic anemia N	Patients with chromosomal abnormalities N	Chromosome 7 N	Trisomy 8 N	Remarks
Appelbaum [4]	176	7	3	2	
De Planque [21]	38	5	3	–	
Mikhailova [32]	69	18	2	8	
Jameel [22]	31	7	NA	4	
Kojima [65]	113	12	6	7	Children
Fuhrer [35]	114	7	2	1	Children
De Planque [25]	468	12	–	–	
Kaito [9]	72	5	4	1	
Maciejewski [14]	189	29	11	6	
Rosenfeld [41]	122	13	10	1	

only 7 cases was the karyotypic abnormality known not to be present initially [32].

The evolution of an abnormal karyotype has been also reported in children, as in series of 114 pediatric patients from Europe, 7 patients developed chromosomal abnormality but the aberrant clone was retrospectively found in 2 of them at presentation [35], decreasing the true evolution rate in this study to 5/114. Among 40 Japanese pediatric patients treated with G-CSF and CsA, 11 showed clonal evolution, mainly to monosomy 7 [10]. Recently, the same group reported development of MDS or AML in 5 out of 41 children treated with immunosuppression for their hepatitis-associated AA, indicating that different etiologies may result in a final pathophysiologic pathway leading to clonal evolution [36].

After clonal evolution, marrow morphology was characterized by predominance of hypercellularity (41%) and patchy biopsy patterns (27%), while continued hypocellularity was found in 1/3 of the patients. Frank dysplasia, including changes in megakaryocyte morphology, was found in 15 out of 29 patients, and a left shift in myeloid differentiation was observed in 12. However, in 9 out of 29 patients there were no morphologic changes suggestive of MDS [14]. While the entity of AA with cytogenetic abnormalities may exist, new appearance of a karyotypically abnormal clone in the course of AA warrants the change of current diagnosis of AA to MDS. In the recent NIH analysis, patients with AA were followed with periodic cytogenetic analyses of marrows, and evolution of abnormal karyotypes was identified in 29 patients (a total of 189 patients were analyzed), allowing for the estimation of the evolution rate of 14% in 5 years and 20% in 10 years, respectively. The time to evolution varied, and it appears that there is a slow and steady acquisition of chomosomal damage [14]. Of note is that, while the detection of a new cytogenetic abnormality is a stringent diagnostic sign, it may not reflect the total rate of MDS evolution in AA. In primary MDS, the proportion of patients with a normal karyotype is 40–60%, and by analogy, it is possible that also post AA, MDS can evolve without an overt chromosomal damage. Clearly, an elevated number of blasts and hypercellularity unmistakably herald the evolution to MDS, but such obvious presentation is not common. Clinical signs of evolution include recurrence of cytopenia after initial improvement, primarily refractory course or be even less obvious and certain types of chromosomal abnormalities were associated with distinctive clinical features (see below), but it is clear that simple extrapolation from the data in primary MDS for which larger series exist are not possible [14].

Risk Factors

As various types of MDS and clonal abnormality may have different underlying pathophysiologies, it may be difficult to identify specific risk factors for progression to clonal disease. For example, monosomy 7 appears to evolve in primary refractory patients or those with incomplete response to immunosuppression, while trisomy 8 was observed in patients whose counts improved adequately [14]. Similar clinical observation was made for the 13q- abnormality [37]. In another study, a similar distribution of monosomy 7 between responders and non-responders was observed [35]. In contrast to ATG, cytoxan was described to prevent cytogenetic evolution, but at least one patient who received cytoxan was reported to later develop an abnormal karyotype [38]. In a randomized trial, no differences between ATG and CsA with respect to occurrence of cytogenetic abnormalities have been found, due to the low number of patients enrolled [39].

Types of Clonal Defects in AA

The most commonly found cytogenetic abnormalities following AA were aberrations of chromosome 7 and trisomy 8. For example, in a study from Seattle, monosomy 7 was found in 3 and trisomy 8 in 2 of 7 patients with karyotypic abnormalities [4], and in a Dutch series, in 3 of 5 AA patients who evolved to MDS [21]. Among Italian patients, trisomy 8 was most frequent, present in 8 out of 18 cases, followed by monosomy 7 in 2 out of 18 patients, but only a minority of these patients had normal cytogenetics at the time of presentation [32]. In Pakistan, trisomy 8 was found among 7 abnormal karyotypes in otherwise typical AA patients [22]. In the NIH series of 29 patients, trisomy 8 was present in 7 patients and aberrations of chromosome 7 in 12 patients. In contrast to these results, a recent meta-analysis showed trisomy 6 as most common in AA [29], but in all of the patients included in this study, the abnormal karyotype was present at initial diagnosis and most proved refractory to immunosuppression, suggesting that marrow hypocellularity may have precluded the alternative diagnosis of MDS. In a recent report from Japan, a series of 9 patients with 13q- following otherwise typical AA was reported [37]; in the NIH experience, 13q- was also reported in several patients of the 29 patients who developed an abnormal karyotype after AA [14]. In agreement with the Japanese report, both patients showed stable counts and a good response to immunosuppression. In a study of children in Japan, monosomy 7 occurred at the highest frequency [40], and in children reported from Germany and Austria, monosomy 7 was present in 2 out of 7 patients while trisomy 8 was encountered once [35]. In a long-term update of the NIH ATG/CsA trial, aberrations of chromosome 7 was present in 10 and trisomy 8 in 2 out of 13 patients [41]. All other abnormalities appear to occur more randomly and given the overall low number of patients reported, it is difficult to establish individual frequencies.

There are no predictive factors to identify patients at risk for the clonal evolution of myelodysplasia. No significant differences were found between the ages at presentation of AA, time to evolution of cytogenetic

abnormality, or blood counts at presentation (although AA patients who later developed monosomy 7 showed a trend to a lower initial ANC). The response to immunosuppression does not correlate with a smaller risk of cytogenetic evolution; in the NIH study, out of 29 patients who developed clonal abnormality, there were 12 patients who did not respond to immunosuppression and 16 responders, a ratio not different from that seen among all patients. The prevalence of PNH clone detection in patients who develop abnormal cytogenetics in this group of patients was at least 48% [14].

In retrospect, blood counts of patients at the time of the cytogenetic evolution to trisomy 8 were significantly higher than those of patients with monosomy 7. Additionally, even after evolution to trisomy 8, sustained improved blood counts were often dependent upon continued CsA administration. Patients with monosomy 7 and those with complex karyotypes usually (but not always) had a poor response to immunosuppression and persistent pancytopenia [14]. When compared with patients with numerical or structural defects of chromosome 7 at diagnosis of abnormal karyotype, those with trisomy 8 showed a significantly higher neutrophil counts, hemoglobin concentration, mean reticulocyte and platelet count at the time of chromosomal evolution. In contrast, trisomy 8 and other prognostically more benign abnormalities were frequently seen in the context of a good hematologic response to immunosuppressive therapy [14,42]. Similar clinical behavior was observed in patients with 13q- [37]. In agreement with this observation, responses have been observed in patients with AA and cytogenetic abnormalities; in 6 patients with diverse abnormalities and hypocellular bone marrow, a response to ATG and CsA has been observed in 4 of them [40].

Although the appearance of a cytogenetic abnormality in a patient with AA is strong evidence of clonal evolution to MDS, in some studies, a high proportion of apparently transient chromosomal changes would diminish the diagnostic and prognostic implications of new cytogenetic findings. Conversion to a normal karyotype is not a frequent event and may also be a function of the frequency of marrow exams. In our previously published study [14], such an event was observed in only 2 out of 29 patients, but since publication of this report another patient reverted (unpublished observation). It is likely that abnormal clones may be recruited and to contribute to blood production for limited periods of time.

Comparison of MDS Secondary to AA and Primary MDS

MDS, evolving from AA, and primary MDS differ in the distribution of specific cytogenetic abnormalities. In primary MDS, aberration of chromosome 5 are generally cited as the most frequent, present in 10–37% of all patients [43–48], but this chromosome is only rarely affected in AA patients. 20q- and -Y also are more often abnormal in primary MDS in comparison to AA [43,45]. Conversely, monosomy 7, most prominent in the late evolution of MDS from AA, occurs in a minority of primary MDS, 6.5–11% [43,46–51]. Trisomy 8 appears to have a comparable incidence among cytogenetic abnormalities evolving from AA and in primary MDS (6–20%; [43–48,50,51]).

Specific cytogenetic abnormalities are strong predictors of clinical behavior and survival in hematologic disease, including acute and chronic leukemias [52,53] and primary MDS [54]. In most of the studies, in agreement with the IPSS classification for primary MDS [54], there was a major difference in the prognosis of patients who developed defects of chromosome 7 or who had complex karyotypic abnormalities: these lesions were present in most of our patients who died and/or developed leukemia. The prognosis of trisomy 8 in primary MDS is not entirely favorable; in the IPSS, trisomy 8 is included in the intermediate risk group with an average survival of 2.4 years (17.2 months in analysis of 115 patients with trisomy 8; [50]) and time to evolution of AML of 1.6 years [54]. In other studies, trisomy 8 has been found in more advanced FAB subtypes and associated with a high risk of leukemic evolution, with survival as poor as attributed to monosomy 7 [44,48,50,51,55]. The sharp contrast of these clinical data with the clinical course and prognosis of trisomy 8 evolving from AA suggests a different biology for an apparently identical cytogenetic abnormality in late AA and in primary MDS. Patients with abnormalities of chromosome 7 in AA fared as poorly as in primary MDS, with a high rate of conversion to acute leukemia [51,54].

Clinical similarities between MDS and AA are most obvious in the hypocellular form of dysplasia, and clinical distinction is often not possible. Few characteristics were reported to be more typical of hypocellular MDS than AA and included hypochromasia hypogranulation, blasts, ring and pelgeroid neutrophils and, perhaps most distinctive, circulating micro-megakaryocytes [56]. When in one study, clinical features of patients hypo- vs. normo/hypercellular MDS were compared, no major differences in survival, transformation karyotypes or FAB subtypes were found between the groups [57]. However, in other reports, contrasting observations were reported with a longer survival and a lower rate of transformation to AML [58,59]. Therefore, it is likely that hypocellular MDS may be as heterogenous with regard to the clinical features and pathophysiology as the typical MDS [56,59].

Per clinical inference to AA [17], immunosuppressive therapy has been applied to patients with MDS, especially those with hypoplastic form [6–8,60–62]. Overall, the results were somehow conflicting with regard to both CsA and ATG [63], but in the NIH studies, features reminiscent of a typical AA, such presence of HLA-DR15 and PNH clone were found to be associated with a better chance of response to immunosuppression [7,64]. While hypocellular forms of MDS may also be suggestive of potential responsiveness to immunosuppressive ther-

apy, responses have been described in patients with normo- and hypercellular marrow as well as in patients with cytogenetic abnormalities, but clearly, more advanced stages of MDS are less likely to respond to immunosuppression.

Prognosis

The diagnosis of MDS in the course of AA has prognostic significance. Most obvious modifiers include the presence of blasts, hypercellular bone marrow, certain types of defects, and recurrence or persistence of profound cytopenia, all constituting unfavorable prognostic markers. For example in one report, AA patients, who developed secondary chromosomal abnormalities, showed a mortality rate of about 27% with a mean follow up after evolution of 29 months (from the initial diagnosis, the total observation interval was 70 months). All but two deaths were related to complications of leukemia. In total, 13 patients (45%) developed either RAEBt or AML. Of these patients, 3 underwent unrelated matched bone marrow transplant [14].

Response to immunosuppression in patients with aplasia and abnormal karyotype may be as high as 50% [30], and certain karyotypic abnormalities (trisomy 8, 13q-) may favorably respond to immunosuppression. While low numbers of patients reported preclude generalization, no individual abnormality predicted unresponsiveness. However, certain types of chromosomal defects are less likely to benefit from immunosuppression, including monosomy-7, complex karyotypes or 5q-syndrome, and bone marrow transplantation may be the only therapeutic option for patients affected.

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