Therapy-related MDS: the importance of repeating cytogenetics and immunophenotyping in “relapsed” AML

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Abstract Therapy-related myelodysplastic syndromes (t-MDS) occurring after the successful treatment of acute myeloid leukaemia (AML) are rare and may be difficult to distinguish from relapse of the primary leukaemia, especially if the second neoplasm overlaps morphologically with the initial one. We report a 78-year-old male who, 3 years after treatment for acute monocytic leukaemia, developed t-MDS (refractory anaemia with excess blasts-2) that was initially suspected to be a relapse. Cytogenetic analysis of the initial AML had revealed +8 and del(16). These abnormalities disappeared, along with monocytic markers on flow cytometry, 3 years later, and a new t(3;7) event was found instead. The cytogenetic findings, together with the clinical profile, the morphological data and the immunophenotypic shift, were crucial in correctly recognizing the t-MDS and excluding relapse. Repeat genetic and immunological analyses are

often omitted at the time of morphologically evident relapse in AML, especially for economic reasons in resource-constrained settings. Our case shows that such investigations may be invaluable in avoiding a misdiagnosis and, indeed, may help reveal the true incidence of t-MDS/t-AML in treated AML patients.

Keywords Acute myeloid leukaemia · Cytogenetic analysis · Flow cytometric immunophenotyping · Late complications · Secondary malignancy · Therapy-related myelodysplastic syndromes

Introduction

Therapy-related acute myeloid leukaemia/myelodysplastic syndromes (t-AML/MDS) are well-recognized events in patients who have received potentially genotoxic therapy, usually for prior malignancies or autoimmune disorders [1–6]. Their outcomes have historically been poor in comparison to persons who develop de novo AML. In recent times though, it has been questioned whether a diagnosis of t-AML per se indicates a poorer prognosis or whether the adverse outcomes result from other distinctive clinical/biological characteristics [3, 4].

The occurrence of t-AML/MDS after treatment of AML is a rare event. A University of Chicago series of 306 patients [5], for instance, included no cases that followed AML. The commonest primary diagnoses were solid tumours and Hodgkin and non-Hodgkin lymphomas [5]. It is possible that t-MDS/AML may be under-recognized by hematopathologists in patients with a prior history of treatment for AML due to an assumption of relapse. The diagnosis may be especially challenging if the entity is unsuspected, as the second malignancy is likely to overlap morphologically with the initial one. We report one such case of t-MDS where repeat cytogenetic and immunophenotypic analyses were crucial to avoiding a misdiagnosis of relapsed AML.

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Case report

A 75-year-old male presented in March 2007 with progressive weakness and gum bleeds lasting 15 days along with high-grade fever and bilateral chest pain for the last 2–3 days. He had previously enjoyed good health, and a routine medical check-up 3 months ago (December 2006) had recorded normal blood counts. He gave a remote past history of iritis in the left eye in 2001 treated presumptively but successfully with anti-tubercular drugs for 6 months and Herpes zoster treated with oral acyclovir in 2005. He had received oral methotrexate 40 years ago for approximately 7 days for psoriasis that had otherwise been controlled with topical salicylates and steroids. On examination, there was fever and mild pallor with gum hypertrophy and bilateral crepts in the chest. No organomegaly or lymphadenopathy was detected.

Investigations revealed haemoglobin (Hb) 114 g/l, total leukocyte count (TLC) 112×10^9 per litre, platelets 34×10^9 per litre with mean corpuscular volume (MCV) 87.1 fl. A peripheral smear showed marked leucocytosis with 29 % blasts and 40 % promonocytes. A bone marrow (BM) aspirate was hypercellular and showed 27 % blasts with 34 % promonocytes (i.e. total blasts and blast equivalents 61 %) along with 10 % abnormal/immature monocytes (non blast-equivalent monocytoid cells with chromatin condensation but no nucleoli in indented to lobulated nuclei and variably basophilic cytoplasm with frequent granules and/or vacuoles) (Fig. 1). No Auer rods or Phi bodies were seen. Dysplastic changes in the few residual granulocytic and erythroid cells in the blood and bone marrow did not reach the requisite ≥ 50 % criterion [1] for AML with myelodysplasia-related changes. Cytochemical stain for α -naphthyl butyrate esterase was strongly positive, while myeloperoxidase was negative in the blasts and promonocytes. Flow cytometric analysis on the

marrow demonstrated a prominent blast and promonocyte cluster positive for CD4 (58 %), CD11c (moderate, 62 %), CD13 (moderate, 82 %), CD14 (bright, 62 %), CD33 (bright, 86 %), CD34 (dim, 65 %) and HLA-DR (bright, 57 %). Lymphoid markers tested (CD2, CD5, CD10, CD19, CD20 and cytoplasmic CD79a and CD3) were negative. Conventional cytogenetic analysis of the marrow revealed 46XY, del(16q22)[15]/47XY,+ (8)[5].

A diagnosis of acute myeloid leukaemia (AML) with monocytic differentiation [FAB subtype AML M5b] with intermediate risk cytogenetics (as per the Revised MRC Prognostic Classification, [7]) in an elderly male was made. The possibility of AML with myelodysplasia-related changes was also considered because of his age. However, his previously excellent physical condition, the absence of morphological dysplasia or the requisite cytogenetic abnormalities included in the WHO classification [1], and significantly, the normal range blood counts just 3 months prior to the diagnosis of AML strongly argued against a background of MDS.

The patient received an induction regimen of subcutaneous low-dose cytosine arabinoside (AraC) for 10 days. He attained a complete remission with normalization of counts. Subsequently, he received maintenance therapy comprising of nine cycles each of daunomycin and AraC alternating with Ara C and 6-mercaptopurine for 5 days each for the next 18 months. Supportive care comprised of blood transfusions, growth factors and antibiotics as required. He remained largely complaint-free for 3 years, only getting tri-monthly blood counts done.

In October 2010, while he remained asymptomatic with no significant examination findings, a follow-up hemogram showed Hb 106 g/l, TLC 43.3×10^9 per litre, platelets 32×10^9 per litre and an MCV of 109.4 fl. The peripheral smear showed leukocytosis with 4 % blasts and many hypolobate and hypogranular neutrophils (Fig. 2). The absolute

Fig. 1 Bone marrow aspirate in March 2007 showed 27 % blasts (depicted in figure) and 34 % promonocytes. The inset shows promonocytes in the peripheral blood (Wright–Giemsa, $\times 1,000$)

Fig. 2 Peripheral smear in October 2010 showed circulating myeloid precursor cells. Four percent blasts were seen (inset). Relapse was suspected. (Wright–Giemsa, $\times 400$)

Fig. 3 Bone marrow aspirate in October 2010 showed granulocytic preponderance with 11 % blasts. Hypogranular myeloid precursors and a few hypolobate neutrophils are present (Wright–Giemsa, $\times 1,000$). *Inset* shows a dysplastic megakaryocyte (Wright–Giemsa, $\times 400$)

monocyte count was 0.85×10^9 per litre. Relapse was suspected, and a BM aspirate was done. The marrow was markedly hypercellular with a myeloid preponderance and showed 11 % blasts with significant dysgranulopoiesis in the form of hypogranular myelocytes and cells with hypolobate and ring nuclei comprising 35 % of series (Fig. 3). Megakaryocytes were diminished, and only dysplastic forms with hypolobate nuclei and micromegakaryocytes were seen (Fig. 3, inset). There was a marked paucity of erythroid cells. Conventional cytogenetics this time on the bone marrow revealed 46XY, t(3;7)(q22;26)[20]. The initial abnormalities of +8 or del(16q22) were absent (Fig. 4). Flow cytometry revealed a progenitor region cluster (~10 % of all events) that was positive for CD13, CD15, CD33, CD117 and CD34 but

was negative for all monocytic markers (CD4, CD11b, CD11c, CD14 and CD64) as well as for MPO.

The differential diagnoses entertained were a relapse of his AML versus t-MDS (therapy-related refractory anaemia with excess blasts, t-RAEB-2). Taking into consideration the history of chemotherapy, the dysplastic morphology, the absence of any monocytic markers on this blast cluster and the emergence of a new cytogenetic aberrancy coupled with the absence of previous cytogenetic abnormalities, t-RAEB-2 was the likeliest diagnosis [1]. His Age-Adjusted Revised International Prognostic Scoring System (IPSS-RA) score [8] was >6 , indicative of very high-risk MDS (although it must be noted that the IPSS-R is validated for and therefore most applicable to de novo MDS). On discussing treatment options, he chose not to undergo aggressive chemotherapy and was started on hydroxyurea 1.5 g/day and lenalidomide 5 mg/day administered at a local centre with monitoring of blood counts. He deteriorated progressively and finally succumbed to the disease 3 months after the bone marrow diagnosis.

Discussion

Therapy-related myeloid neoplasms comprise a distinct subgroup in the 2008 WHO classification of AML [1]. Although cases may be sub-classified as t-AML, or as t-MDS or t-MDS/MPN depending on the blast count, they are considered a single biologic disease with similar genetic features [1, 5, 9]. Since most t-AML/t-MDS patients have previously received alkylating agents and topoisomerase II inhibitors and/or radiation, a division according to the type of therapy (alkylating agents or topoisomerase II inhibitors) is usually unfeasible and is not recommended anymore [1].

Fig. 4 The karyotype in October 2011 reveals t(3;7)(q22;26)

Our patient had a translocation involving chromosome 7, a finding typically previously associated with alkylating agent-related t-AML/t-MDS. His multilineage dysplasia, blast percentage <20 % and subsequent rapidly downhill course were compatible with this entity, while the relatively short latent period of 3.5 years and the abrupt onset were anomalous [1, 2]. This simply reiterates the fact that significant overlaps occur between alkylating agent and topoisomerase II inhibitor-associated t-AML/t-MDS and possibly both contributed to the second malignancy in him.

Over 90 % of patients with t-AML/t-MDS possess cytogenetic abnormalities identical to those found in AML with myelodysplasia-related changes or in de novo AML with recurrent genetic abnormalities [5, 10]. However, except for some patients with t-AML associated with inv(16), t(16;16) or t(15;17), patients with therapy-related myeloid neoplasms appear to have significantly inferior outcomes than their de novo counterparts, suggesting important biologic differences [1, 5, 11, 12]. Investigations are ongoing into whether t-AML's prognosis is inferior when matched for cytogenetic risk group with de novo AML. A recent data suggests that the poor prognosis of at least radiation-related t-AML may be largely dependent on the higher incidence of adverse cytogenetic events [3, 4].

The availability of cytogenetic and immunophenotypic studies both at diagnosis as well as at suspicion of relapse in our patient helped distinguish relapse from a secondary malignancy. In a resource-constrained setting, the subsequent investigations may simply not have been sent once circulating blasts were documented in a blood film and relapse might have been assumed. Data on cytogenetics anomalies in relapsed AML is scarce [13, 14]. When compared with cytogenetics at initial diagnosis, the same karyotype is found at relapse in 40–50 % (20–30 % show both normal karyotypes and ~20 % the same aberrant karyotype at diagnosis and at relapse). Nearly 15–20 % patients have a normal karyotype at diagnosis and acquire an aberrant one at relapse. Of those with an aberrant karyotype at diagnosis, 25 % gain additional chromosomal aberrations at relapse [13, 14]. Only 2–5 % patients show a completely new clone at relapse that is unrelated to the clone at diagnosis. It has been suggested that secondary AML rather than relapsed primary AML should be suspected in these rare persons [15]. For instance, Kern et al. [6] found only 1 of 117 patients, while Garson et al. [13] found 5 of 103 patients to have a completely different karyotype from diagnosis. In both studies, these patients (with abnormal as well as unstable karyotypes) had the worst outcomes of all cytogenetic groups. It must be noted, however, that distinction between t-AML/MDS versus relapsed AML may currently only have limited therapeutic implications in an elderly patient since both portend particularly poor outcomes [6, 9, 13–15].

This case illustrates the multi-modality diagnostic approach adopted by the WHO classification of AML wherein

morphology is only one of the criteria used to arrive at the diagnosis. Although time consuming and expensive, the addition of immunological and genetic data helps classify these often overlapping entities into biologically distinct and therapeutically relevant subgroups [1]. The secondary genetic locus in our patient, for instance, has previously been implicated to result in overexpression and dysregulated transcription of the *EVII* protooncogene in patients with t(3;7)(q26;q21) translocated AML and blast crisis of chronic myeloid leukaemia [16].

In conclusion, accurate diagnosis of t-MDS requires awareness of the entity, knowledge of its biology and behaviour and a high index of suspicion. The last is best aroused when the complete clinical background and the results of ancillary investigations are available to the reporting hematopathologist. Repeat cytogenetic and immunophenotypic evaluation can yield diagnostically useful information and are thus indicated even at morphologically documented relapse in AML patients.

Ethics statement All ethical standards laid down by the Declaration of Helsinki (1964) and its later amendments have been followed in this manuscript.

Conflict of interest The authors declare no conflicts of interest, whether financial or otherwise.

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (2008) WHO classification of tumours of haematopoietic and lymphoid tissues 4th edn. IARC, Lyon
2. Tyagi S, Sharma P (2009) Secondary Leukaemias. In: Choudhry VP, Saxena R, Pati HP (eds) Recent Advances in Hematology, 3rd edn. Jaypee Brothers Medical Publishers, New Delhi, pp 211–225
3. Nardi V, Winkfield KM, Ok CY, Niemierko A, Kluk MJ, Attar EC, Garcia-Manero G, Wang SA, Hasserjian RP (2012) Acute myeloid leukemia and myelodysplastic syndromes after radiation therapy are similar to de novo disease and differ from other therapy-related myeloid neoplasms. *J Clin Oncol* 30:2340–2347
4. Larson RA (2012) Cytogenetics, not just previous therapy, determines the course of therapy-related myeloid neoplasms. *J Clin Oncol* 30:2300–2302
5. Smith SM, Le Beau MM, Huo D, Karrison T, Sobecks RM, Anastasi J, Vardiman JW, Rowley JD, Larson RA (2003) Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood* 102:43–52
6. Kern W, Haferlach T, Schnittger S, Ludwig WD, Hiddemann W, Schoch C (2002) Karyotype instability between diagnosis and relapse in 117 patients with acute myeloid leukemia: implications for resistance against therapy. *Leukemia* 16:2084–2091
7. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, Wheatley K, Harrison CJ, Burnett AK (2010)

- National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* 116(3):354–365
8. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, Bennett JM, Bowen D, Fenaux P, Dreyfus F, Kantarjian H, Kuendgen A, Levis A, Malcovati L, Cazzola M, Cermak J, Fonatsch C, Le Beau MM, Slovak ML, Krieger O, Luebbert M, Maciejewski J, Magalhaes SM, Miyazaki Y, Pfeilstöcker M, Sekeres M, Sperr WR, Stauder R, Tauro S, Valent P, Vallespi T, van de Loosdrecht AA, Germing U, Haase D (2012) Revised international prognostic scoring system for myelodysplastic syndromes. *Blood* 120(12):2454–2465
 9. Singh ZN, Huo D, Anastasi J, Smith SM, Karrison T, Le Beau MM, Larson RA, Vardiman JW (2007) Therapy-related myelodysplastic syndrome: morphologic subclassification may not be clinically relevant. *Am J Clin Pathol* 127:197–205
 10. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A, Bloomfield CD (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 114:937–951
 11. Andersen MK, Larson RA, Mauritzson N, Schnittger S, Jhanwar SC, Pedersen-Bjergaard J (2002) Balanced chromosome abnormalities inv(16) and t(15;17) in therapy-related myelodysplastic syndromes and acute leukemia: report from an international workshop. *Genes Chromosomes Cancer* 33:395–400
 12. Rowley JD, Olney HJ (2002) International workshop on the relationship of prior therapy to balanced chromosome aberrations in therapy-related myelodysplastic syndromes and acute leukemia: overview report. *Genes Chromosomes Cancer* 33:331–345
 13. Garson OM, Hagemeijer A, Sakurai M, Reeves BR, Swansbury GJ, Williams GJ, Alimena G, Arthur DC, Berger R, de la Chapelle A et al (1989) Cytogenetic studies of 103 patients with acute myelogenous leukemia in relapse. *Cancer Genet Cytogenet* 40:187–202
 14. Estey E, Keating MJ, Stass S (1995) Change in karyotype between diagnosis and first relapse in acute myelogenous leukemia. *Leukemia* 9:972–976
 15. Ludwig WD, Haferlach T, Schoch C (2003) Classification of Acute Leukemias (Perspective 1). In: Pui CH (ed) *Treatment of Acute Leukemias: New Directions for Clinical Research*. Humana Press, Totowa, New Jersey, USA, pp 3–41
 16. Storlazzi CT, Anelli L, Albano F, Zagaria A, Ventura M, Rocchi M, Panagopoulos I, Pannunzio A, Ottaviani E, Liso V, Specchia G (2004) A novel chromosomal translocation t(3;7)(q26;q21) in myeloid leukemia resulting in overexpression of EVI1. *Ann Hematol* 83:78–83