Case study

Diagnosis of Therapy-related Acute Myeloid Leukemia with t(8;21)(q22;q22.1) After Treatment for Mantle Cell Lymphoma and Oral Squamous Cell Carcinoma

ABSTRACT

Aims: We report a rare case of therapy-related AML with t(8;21)(q22;q22.1) that occurred after treatment for mantle cell lymphoma (MCL) and oral squamous cell carcinoma (OSCC). **Presentation of case:** A 52 years-old male patient was diagnosed with MCL in leukemic phase. The treatment consisted in R-CHOP rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone, then patient experienced remission. Three months later, he presented a lump that was diagnosed as OSCC, which was surgically removed and treated with cisplatin and radiotherapy. Then, the patient's hemogram presented 35.0% of blasts and, after morphologic, phenotypic and molecular analysis, it was classified as AML with t(8;21)(q22;q22.1). However, due to the previous historic of chemotherapy and radiotherapy, the final diagnosis was t-AML. **Discussion:** The correct diagnosis of therapy related malignancies is important due to its severity as they are very aggressive and, usually, considered incurable. t-AMLs with t(8;21)(q22;q22.1) is considered as favorable karyotype, still, it has a poorer outcome compared with its *de novo* counterpart. **Conclusion:** t-AML with t(8;21)(q22;q22.1) is rare and few cases are described in the literature. More reports are necessary to better elucidate the mechanisms involved in this disease to define better treatment strategies to prevent these events and to improve the poor outcomes.

Keywords: Therapy-related neoplasms, mantle cell lymphoma, oral squamous carcinoma, t-AML.

1. INTRODUCTION

According to the *Classification of Tumours of Haematopoietic and Lymphoid Tissues* by the World Health Organization (WHO), therapy-related myeloid neoplasms (t-MNs) are a distinct class of hematological malignancies that occur after cytotoxic chemotherapy and/or radiation therapy (RT) administered for a previous neoplastic disorder. t-MNs include therapy-related acute myeloid leukemia (t-AML), myelodisplastic syndromes (t-MDS) and myelodisplastic/myeloproliferative neoplasms (t-MDS/MPN). These diseases carry high-risk karyotypes and have a significantly poorer outcome when compared with *de novo* hematopoietic malignancies [1,2].

t-MNs are initiated by mutations or changes in hematopoietic stem cells and/or in the bone marrow (BM) microenvironment as a consequence of cytotoxic treatments or the selection of a myeloid clone with a mutate phenotype. The most commonly cytotoxic agents implicated in t-MNs are alkylating agents (AA) (such as cyclophosphamide and cisplatin), topoisomerase II inhibitors (TPI) (like etoposide and doxorubicin), ionizing RT, antimetabolites and antitubulin agents (such as vincristine) [1]. The incidence of t-MNs is

expected to raise due to the increased survival rates of cancer patients. In fact, there are nearly 12 million cancer survivors today only in the United States [3]. Therefore, considering the poor outcome of t-MNs, the correct diagnosis of these malignant disorders is crucial to better assist the patients.

It is known that t-AML with cytogenetic abnormalities such as t(8;21), inv(16) and t(15;17) have significantly better outcomes than other t-MNs, however, these cases are uncommon and represent only 10% of all t-MNs [4]. AML with t(8;21)(q22;q22.1) usually has a favorable prognosis, nevertheless, even AMLs with favorable karyotypes have a slightly poorer outcome compared with their *de novo* counterparts [2]. In this study we report a rare case of t-AML with t(8;21)(q22;q22.1) that occurred after treatment for mantle cell lymphoma (MCL) and oral squamous cell carcinoma (OSCC). The Research Ethics Committee of the Federal University of Santa Catarina approved this study (CAAE: 61598816.7.0000.0121).

2. PRESENTATION OF CASE

A 52 years-old male patient diagnosed with MCL was admitted to the University Hospital Professor Polydoro Ernani de São Thiago (HU-UFSC) for lymphoma staging and to start chemotherapy treatment. The patient's blood smear analysis revealed a predominance of small cells with a high nucleus/cytoplasm ratio and slight nuclear chromatin condensation, whereas some of these cells also presented cleaved nucleus (Figure 1A). In order to confirm a possible peripheral blood (PB) involvement, immunophenotyping by flow cytometry was required. The analysis presented 78.1% of lymphoid B (CD19+) mature (CD20+, CD45++) cells, with low FSC and SSC, an aberrant expression of CD5 and no expression of CD10, CD23 and CD200. Among these cells, 99% presented lambda light chain restriction. The phenotype of these pathological cells was suggestive of MCL with PB involvement, which characterizes MCL in leukemic phase (Figure 1B-J). The treatment consisted in 8 cycles of R-CHOP rituximab (600mg), cyclophosphamide (1.230 mg), doxorubicin (82 mg), vincristine (1 mg) and prednisone (20 mg) over 5 months. Then, the patient experienced remission.

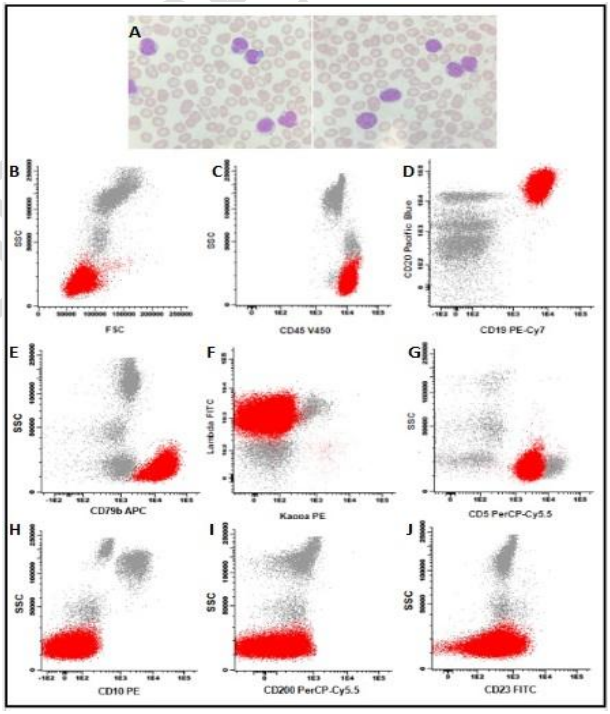

Figure 1 - A) Morphology of PB smear presenting small cells with large nucleus, slight nuclear chromatin condensation, abnormal segmentation and some cleaved nucleus. B-J) Representative dot plots of pathological cells (red population): B) Small cells with low FSC x SSC. C) Expression of CD45. D) Expression of CD19 and CD20. E) Expression of CD79b. F) Lambda restriction. G) Expression of CD5. H) No expression of CD10. I) No expression of CD200. J) No expression of CD23.

About three months after the lymphoma remission, the patient presented a 1.5 cm tumor in the mucosa of the left molar trigone region. The tumor was biopsied and diagnosed as OSCC, which was surgically removed and treated with cisplatin and RT at the Oncology Research Center (CEPON).

One month after the end of OSCC treatment, in a following medical appointment, the patient's hemogram showed 4400 leucocytes/mm³, 35.0% of blast cells, hemoglobin of 6.9 g/dL and a platelet count of 2000 /mm³. The immature cells presented large size, basophilic cytoplasm, slight nuclear chromatin condensation and visible nuclei (Figure 2A); besides, some granulocytic cells showed abnormal nuclear segmentation (pseudo-Pelger-Huët nuclei) (Figure 2B). The immunophenotyping of PB showed 38.60% of blasts (CD34++, CD45+), medium to large sized, committed with the myeloid lineage (MPO+, CD13+, CD33+, CD117+, HLA-DR+) and with aberrant expression of CD19 (70%), CD79a (30%) and CD56 (80%) (Figure 2D-L). The aberrant expression of CD19 in myeloid blasts suggests the presence of t(8;21)(q22;q22.1), which was confirmed by the karyotype and Nested RT-PCR (Figure 2C).

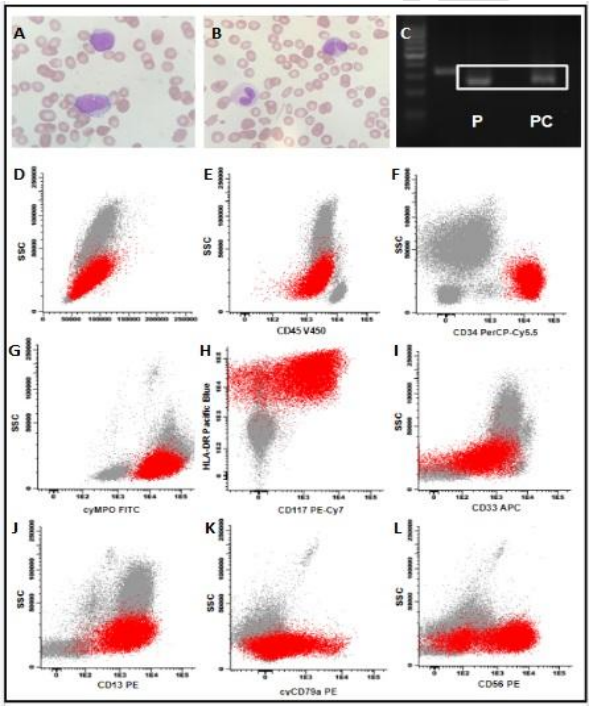

Figure 2. A) Large blasts with high cytoplasm/nucleus ratio, visible nuclei and basophilic cytoplasm. B) Granulocytic cells presenting abnormal segmentation (pseudo-Pelger-Hüet nuclei). C) Nested RT-PCR for t(8;21): P: patient's PB band, PC: positive control band. D-L) Demonstrative dot plots of blasts immunophenotyping (red population): D) Large cells with high FSC x SSC. E) Expression of CD45. F) Expression of CD34. G) Expression of cyMPO. H) Expression of CD117 and HLA-DR. I) Weak expression of CD33. J) Expression of CD13. K) Aberrant expression of CD19 and CD79a. L) Aberrant expression of CD56.

After morphologic, phenotypic and molecular analysis, the disease was classified as AML with t(8;21)(q22;q22.1); however, due to the previous historic of chemotherapy and radiotherapy, the correct diagnosis was t-AML with t(8;21)(q22;q22.1); which has a worse prognosis.

3. DISCUSSION

MCL is a mature B cell neoplasm characterized by PB and/or BM involvement. The correct diagnosis of MCL is important due to its severity as it has been considered incurable, very aggressive and associated with a poor prognosis. The laboratorial diagnosis of MCL is established by the WHO and, overall, the histological confirmation is mandatory. However, the variant morphology observed in MCL can difficult the differential morphological diagnosis between MCL and other B cell neoplasms. Thereby, immunophenotyping important to differentiate between the lymphoma subtypes. Additionally, cytogenetics has a key role in MCL diagnosis by detecting t(11;14), the molecular hallmark of MCL, which is found in more than 95% of cases [1].

According to the WHO, the characteristic MCL immunophenotype includes the expression of B-cell markers (CD19+, CD20+, CD22+, CD79a+, PAX5) and intense IgM/IgD, mostly with lambda light chain restriction. The commonest immunophenotypic markers are CD5+, FMC7+, CD45+, CD43+ and intranuclear cyclin D1+ [1]. In this clinical report, the patient's neoplastic cells presented a classic MCL morphology and immunophenotype (CD19+, CD20+, CD45++, CD5+, CD23-, CD10- and CD200-) (Figure 1A-J).

The adopted treatment regimen was 8 cycles of R-CHOP. According to the literature, cyclophosphamide is an AA and doxorubicin is a TPI, and they are both known to be particularly mutagenic and to have a strong leukemogenic potential [5]. Despite the recent advances in cancer treatment, the currently available chemotherapy regimens, associated or not with monoclonal antibodies, have potential to cause many side effects, including secondary malignant neoplasms. There are many studies that investigate the influence of chemotherapy in the development of leukemia and solid tumors [6,7]. However, the etiology of t-AML and secondary solid cancers after administration of cyclophosphamide and doxorubicin as well as rituximab has not yet been completely elucidated [5].

In this study, about three months after a lymphoma remission, the patient was diagnosed with OSCC, a malignant neoplasm derived from the squamous epithelium of the oral cavity [8]. The lesion was found in the mucosa of the left molar trigone and the diagnosis was determined by histopathology. For OSCC treatment, surgery remains the best option, but chemotherapy and radiotherapy are also applied in combinations to obtain a better response [8]. In this case, the patient's treatment consisted of RT combined with cisplatin; and both methods have potential for the development of AML and SMD [1]. However, one study of meta-analysis [9] found no increased risk of secondary cancers associated with cisplatin compared with non-cisplatin-based chemotherapy. Nevertheless, one month after the end of OSCC treatment, the patient was diagnosed with t(8;21)(q22;q22.1) AML.

AML is a heterogeneous malignancy and cases with t(8;21)(q22;q22.1) represent a group with specific clinical and biological characteristics. The diagnosis of AML with t(8;21)(q22;q22.1) is based on cytomorphology, cytogenetics and immunophenotyping according to the WHO classification. The commonest morphological features include the presence of large blasts with abundant basophilic cytoplasm, sometimes containing azurophilic granules and perinuclear clearing. Some blasts may contain pseudo-Chédiak-Higashi large granules suggesting the presence of the fused gene. Concomitant with the large blasts, some smaller blasts with pseudo-Pelger-Hüet abnormal nuclear segmentation can also be found [1]. These abnormalities were observed in the patient's blood smear (Figure 2B).

The PB immunophenotyping showed myeloid blasts with parcial expression of CD19, CD79a and CD56 (Figure 2J-L), which are lymphoid-associated markers. The expression of CD56 is known to be associated with a poorer prognosis [1]. These results are suggestive of AML with t(8;21)(q22;q22.1) according to the WHO classification, as its characteristic immunophenotypic profile also included a strong expression of CD34, HLA-DR, myeloperoxidase (MPO) and CD13; and a relatively weak expression of CD33. Molecular cytogenetic methods, such as karyotype and PCR, are considered as the gold standard for this malignancy diagnosis, as they allow the identification of t(8;21)(q22;q22.1). In this case report, this translocation was detected by nested RT-PCR (Figure 2C).

Blasts of *de novo* AML with t(8;21)(q22;q22.1) and t-AML share morphological, immunophenotypic, cytogenetic and molecular features, although t-AML with t(8;21)(q22;q22.1) seems to have more dysplastic changes than *de novo* AML [10]. These dysplastic characteristics were observed in the patient's granulocytic cells (Figure 2A-B). Based on these morphologic, phenotypic and molecular analyses and on the previous historic of chemotherapy and RT, this case was finally classified as t-AML.

Studies demonstrated that patients with t-AML with t(8;21)(q22;q22.1) are usually old, have lower white blood cells (WBC) counts and an inferior overall survival than their *de novo* counterparts [2-10]. The patient in this study was 52-years old and had a poor response after treatment with (7+3) cytarabine (100 mg/m^2) and daunorubicin (60 mg/m^2). He presented persistent blasts in the PB and passed away few months after the final diagnosis.

A short latent period without a previous myelodisplastic phase is observed in t-AML with t(8;21)(q22;q22.1) patients and it is associated with prior TPI therapy or RT alone [1]. The mechanisms responsible for such mutations remain unknown, but may involve several chromatin structural elements such as topoisomerase II, which might present preferential breakage sites after exposure to damages such as TPIs [10]. The patient's t-AML was diagnosed four months after the administration of doxorubicin, a TPI, for MCL treatment; and no sign of myelodysplasia was observed before the t-AML diagnosis, which is compatible with the literature description.

4. CONCLUSION

t-AML with t(8;21)(q22;q22.1) is rare and few cases are described in the literature. It is a fatal complication of cancer treatment and its incidence is expected to rise due to the increasing number of cancer survivors. It shares the same morphological, molecular and immunophenotypic features than *de novo* AML with t(8;21)(q22;q22.1), though presenting a worse prognosis. Thus, the correct diagnosis of this disease is crucial due to its severity and low overall survival rates. For that reason, more reports and studies are necessary to better elucidate the mechanisms involved in the development of t-AMLs in order to define better treatment strategies, preventing these events and improving the poor outcomes presented in such cases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

All authors declare that written informed consent was obtained for publication of this case report and accompanying images.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The Research Ethics Committee of the Federal University of Santa Catarina approved this study (CAAE: 61598816.7.0000.0121).

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