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Double Hematological and Solid Malignancy Diagnosed from Bone Marrow Studies: Case Report, Laboratory View

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ABSTRACT

A 63-year-old male patient was evaluated for cytopenia at King Abdullah Medical City Makkah Al-Mukaramah. Complete blood count and bone marrow studies including flowcytometry revealed acute myeloid leukaemia (AML) with dysplastic changes and according to the WHO criteria published in 2018, it was categorised as AML-M2 not otherwise specified. The patient received azacytidine as a sole line of therapy because of his age and general condition. Ten months later, serum ferritin and prolactin level were elevated and the cytopenia became more severe, prompting new bone marrow studies for proper evaluation. Bone marrow metastasis from the existing non-hematopoietic malignant tumour were detected. A diagnosis of metastasis from adenocarcinoma of the lung was made by immunohistochemistry which showed positivity for cytokeratin 7(CK7), pancytokeratin (CKAE1AE3) and thyroid transcription factor (TTF). Additionally, CEA and CA 19-9 were elevated.

To the best of our knowledge, this is the first case of a dual diagnosis of an haematological and a solid tumour in a patient, originating from the Makkah area, Saudi Arabia to be published. Keeping in mind that the patient was treated with azacytidine therapy, which was not licensed for use until now, for the development of secondary malignancies. Further studies for evaluation of its effect are worthy.

1. Introduction

Double primary malignancies could be divided into synchronous and metachronous, depending on the interval between tumor diagnoses [1]. Synchronous malignancy denotes development of the second tumor either simultaneously [2], or within 6 months after the first malignancy [3]. Metachronous malignancies were secondary tumors that have developed after 6 months, or even more than that from the first malignancy.

Metachronous cancers are not rare and it is expected as a side effect of the primary cancer treatment modalities; chemotherapy or radiotherapy [3]. On the other hand, it is rare to find a synchronous dual malignancy at first presentation and it is even rare to detect synchronous solid tumor with a hematological malignancy [3].

Generally, the pathophysiology of malignancies remains poorly understood where many factors seems to play an imminent role such as immunosuppression, genetic predisposition, environmental exposure to carcinogenic factors like tobacco smoking and alcohol consumption [4], infections, and toxic effects related to treatment by chemotherapy or radiotherapy [1]. Where a metachronous malignancy that develops after receiving chemotherapy for another first malignancy could be considered as an iatrogenic, a synchronous dual malignancy is usually related to genetic instability such as DNA microsatellite instability, mutation in multiple tumor suppressor genes such as p16, p53, PTEN and Rb gene [5] or immunosuppression [6]. With modernization of life, the incidence of malignancies has increased globally. With regards to double malignancy, the improved diagnostic modalities and the scientific level of the clinicians contributes greatly to the increased incidence of the diagnosis of such cases not increasing the incidence of the cases their selves.

2. Aim of the report

To alert the medical community about the careful evaluation of patients with known acute myeloid leukemia with special appreciation to the role of the laboratory investigations.

3. Case report

63 years old male patient was presented to King Abdullah Medical City Makkah Al-Mukaramah, KSA. On July 2015. A bone marrow request was sent for proper evaluation of the patient where his complete blood counts revealed both leukopenia and thrombocytopenia.

4. Laboratory tests on admission

4.1. Serum biochemistry

The liver function tests were normal; GGT 87 U/L (Reference range (RR) 15-85), AST 21 U/K (RR 15-37), ALT 43 U/L (RR 16-61), ALP 81 U/L (RR 45-117), Total Bilirubin 0.3 mg/dL (RR 0.2-1), Direct Bilirubin 0.09 mg/dL (RR 0-0.2), A/G ratio 1.6 (RR 1.1-2.2), Albumin 4.6 g/dL (RR 3.4-5).

The kidney function tests were normal; blood urea nitrogen (BUN), 14mg/dL (RR 7-18), Creatinine 0.8 mg/dL (RR 0.4-1.3), Uric acid 5.8 mg/dL (RR 3.5-7.2), Protein 8.1 g/dL (RR 6.4-8.2). Serum electrolyte levels were within normal range, Phosphorus 3.3 mg/dL (RR 2.5-4.9), Mg 1.9 mg/dL (RR 1.6-2.6), K 4.3 mmol/L (RR 3.5-5.1), Na 136 mmol/L (RR 136-145), Corrected calcium 9.5 mg/dL (RR 8.5-10.1),

Viral markers: the patient had negative markers for HCV, HBV, HIV, and H1N1.

4.2. Complete blood count

Peripheral blood cells were examined by an automated hematologic analyzer (Sysmex XN1000). Peripheral blood smear examination showed normochromic anemia and mild rouleaux formation, the red

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cell indices were Hgb 8.8 g/dL MCV 92 fL, MCH 32 pg and MCHC 34. White blood cells showed leukopenia (1.7 x10^9/L). The leukocyte differential count revealed sever neutropenia; neutrophils 22.8%, lymphocytes 69.1%, and monocytes 6.7%, eosinophil 0.7%, basophile 0.7%. Marked thrombocytopenia was confirmed with no clumps or large platelet forms; Platelets count 51 x10^9/L. No blasts were seen.

4.3. Coagulation studies

Prothrombin time (PT) and partial thromboplastin time (PTT) and fibrinogen were within normal range; PT12.2 sec. (RR 11-16), INR 0.9 (RR 0.8-1.2) , PTT 30.5 Sec. (RR 28-44), and fibrinogen 3.2 g/L(RR1.7- 4.2).

4.4. Bone marrow (BM) studies

BM aspiration

Bone marrow smears were stained with Wright-Giemsa and analyzed according to routine clinical laboratory procedures. The BM sample was particulate with cellular particles and trails. Megakaryocytes were adequate in number with few hypolobated forms denoting dysmegakaryopoietic changes. Granulopoiesis was adequate but revealed mainly early granulocytic precursors and some dysplastic changes (degranulation). Active erythropoiesis was noticed with dyserythropoietic changes in the form of binucleated and megablastoid changes. Plasma cells were mildly prominent (2-4%). Some histiocytes were seen but with no evidence of phagocytic activity. The blasts were increased; around 24-30%, and they were variable in size, with large nucleus and multiple nucleoli. The blasts had abundant basophilic granulated cytoplasm. Apart from morphology, more than 5% of the blasts were positive for myeloperoxidase by immunohistochemistry denoting their myeloid nature (Figure 1).

4.5. Bone marrow biopsy:

The BM biopsy revealed 50% cellularity and interstitial involvement by immature looking mononuclear cells; blasts in the background of trilineage hematopoiesis. Some dysplastic megakaryocytes were seen (Figure 1).

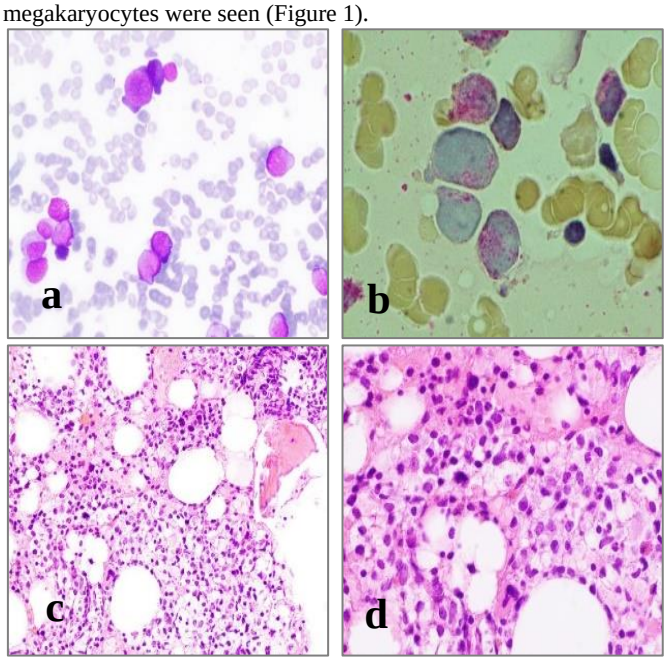

Figure 1: Represents bone marrow (BM) studies at presentation. (a) myeloblasts present in the BM aspiration stained by Wright-Giemsa stain (X 40). (b) Positive myeloperoxidase staining of the blasts. (c & d) BM biopsy reveals normocellular BM with diffuse infiltration by immature cells

4.6. Cytogenetic results

Revealed normal chromosomal count (46, XY [20]) without apparent clonal abnormality. Also, Fluorescence in situ hyperdization (FISH) did not reveal any clonal abnormalities.

4.7. Molecular studies

Revealed negative FLT3, NPM, BCR-ABL, and PML-RARA**.**

4.8. Flowcytometry analysis

Using BD-FACS-Canto II System (BD - Bio Science) and comprehensive panel of monoclonal antibodies labelled with fluorescein stain. The dim CD45 positive cells in blast gated area ranged from 20 to 25%. The blasts expressed the myeloid markers: cytoplasmic MPO, CD13, CD33, CD15, and the stem cell markers CD34 and CD117 and the non-lineage specific marker HLADR in addition to CD9, CD38, and CD58. The blasts were negative to CD11c, CD14, CD16, CD36, CD64, CD66, CD41a, CD2, CD3 surface or cytoplasmic, CD5, CD7, CD8, CD4, CD56, CD10, CD19, CD20, CD22, cytoplasmic CD 79a, TdT, CD25, or CD110. Figure 2 represents some of the positive marker expression.

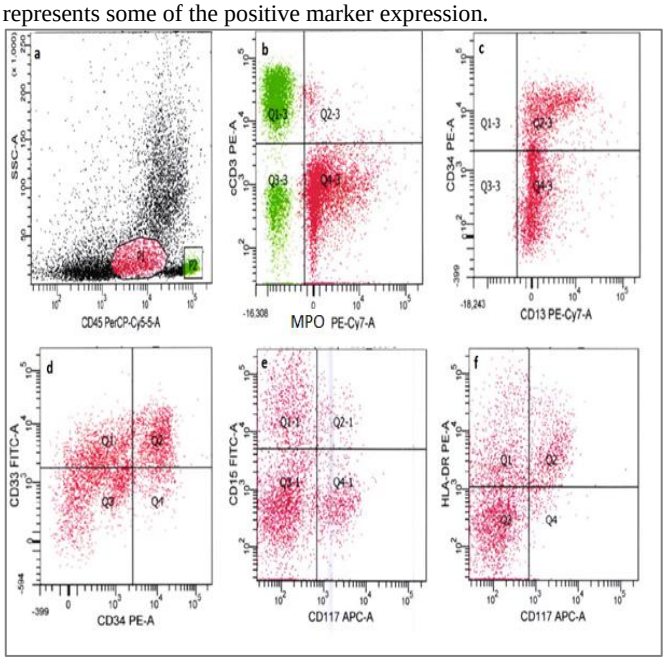

Figure 2: flowcytometry analysis of the BM at presentation. (a) Dim CD45 population; P1 gate represents the leukemic cells. (b) Myeloperoxidase (MPO) expression by the leukemic cells. (c) represents CD13 positive leukemic cells with partial co-expression of CD34. (d) represents CD33 positive leukemic cells with partial co-expression of CD34 (e) CD15 and CD117 expression by the malignant population. (f) Partial expression of HLADR and CD117 by the leukemic population.

The performed bone marrow aspiration / biopsy and immunophenotyping support the diagnosis of acute myeloid leukemia with dysplastic changes and according to the WHO criteria 2018 it is AML-M2 not otherwise specified. Note no WHO reference in list

Being an older patient with generally poor health condition, the only treatment option for this patient was Azacytidine, to reduce the blast count. He did not receive the AML chemotherapy protocol.

The patient was followed up and his investigations were within accepted range for his condition. On May 2016, the serum ferritin level was very high; 2382ng/ml (RR 8-388 ng/ml) and the prolactin level was elevated; 22(0-0.1ng/ml). On the next follow up samples, the cytopenia was very pronounced, necessitating another BM study for better evaluation of the patient condition. The positive laboratory investigations were very high serum Ferritin level; 8109.8 ng/mL, elevated CEA; 48.59 ng/dL (RR 0-5) and CA19-9; 270.08 U/mL (R.R 0-30.9).

In contrast to the PB examination at presentation, the myeloblasts were seen in the PB; 14% and in the BM as well (Figure 3 (b)).

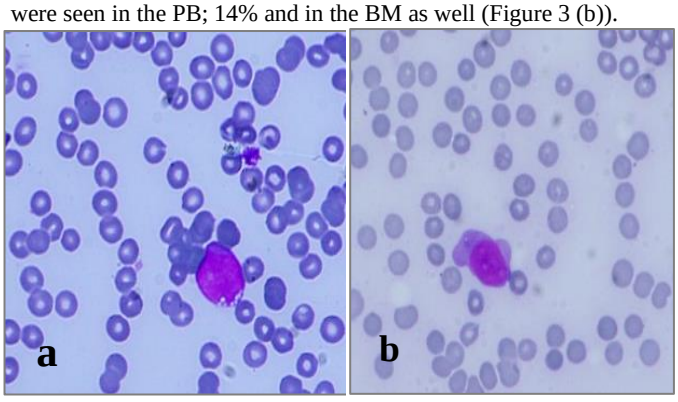

Figure 3: (a) represents the presence of myeloblasts in the peripheral blood. (b) represents myeloblasts in the BM despite being diluted sample.

The examination of the touch print revealed the presence of sheets of malignant non- hematopoietic cells that were obvious also in the BM biopsy with the presence of rosette shaped malignant cells (Figure 4).

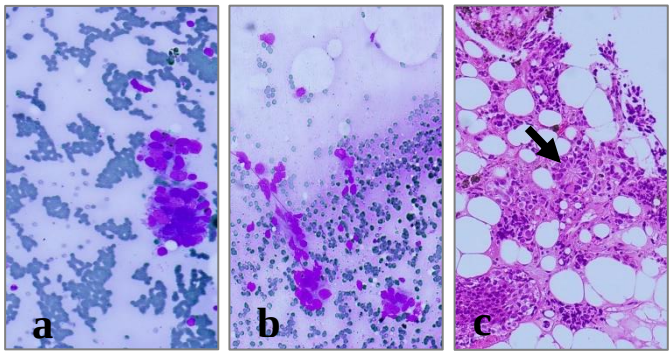

Figure 4: (a) Represents sheets of malignant non-hematopoietic cells in the BM. (b) sheets of malignant non-hematopoietic cells in the touch imprint. (c) the BM biopsy represents malignant non- hematopoietic cells forming rosette (black arrow).

Immunohistochemistry studies on BM biopsy reveals negative reaction for CD45, CD34, prostatic specific antigen (PSA) and anti-IL-7 receptors (CDX2). On the other hand, the malignant non hematopoietic sheets showed positive reaction for cytokeratin 7(CK7), pancytokeratin (CKAE1AE3) and Thyroid transcription factor (TTF) (Figure 5). The expression of TTF is linked to either adenocarcinoma of the lung or the thyroid gland, however the adenocarcinoma of the thyroid gland rarely metastasizes in the BM. Additionally the co-expression of TTF together with CK7 supports the diagnosis of BM secondaries due to lung adenocarcinoma [7].

The patient received palliative therapy and died two months later.

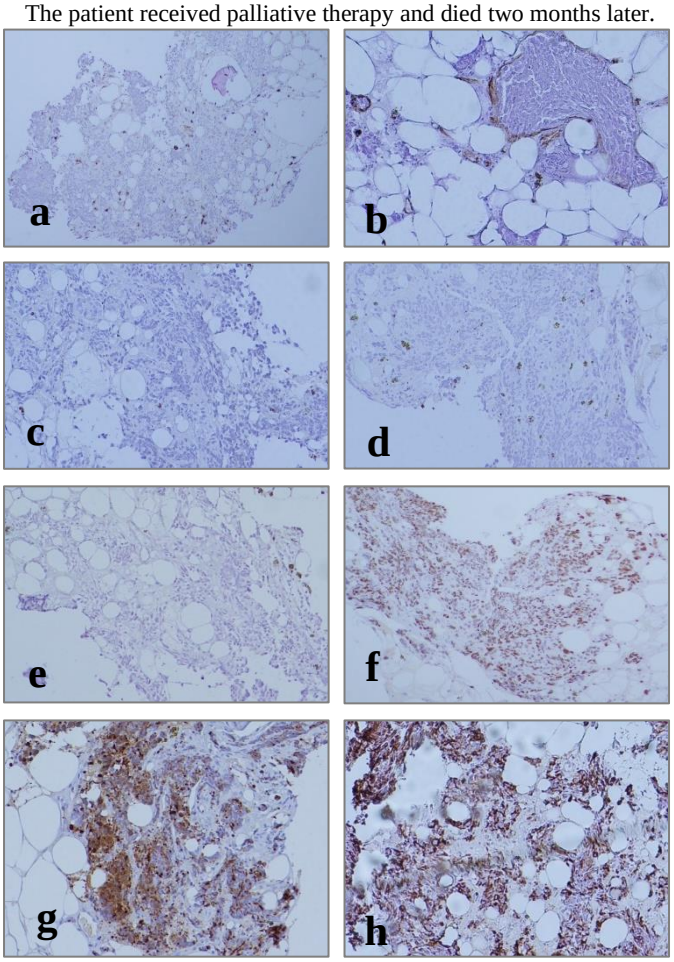

Figure 5: Immunohistochemical staining of the BM biopsy (a) CD45 staining negative. (b) CD34 negative. (c) CD20 negative. (d) anti-interleukin 17 (CDX2) negative. (e) Prostate specific antigen (PSA) negative. (f) Thyroid transfection factor (TTF) positive. (g) cytokeratin 7 positive. (h) Pancytokeratin (CKAE1AE3) positive.

5. Discussion

The criteria used for the diagnosis of multiple primary cancers were first conceived by Warren and Gates in 1932 [8] which stated that:

1. Each of the tumors must be a malignancy confirmed by histology
2. Each must be geographically separate and distinct.
3. Probability of one being the metastasis of the other must be excluded.

These criteria are fulfilled in this case where the AML is confirmed by morphology, IHC and flowcytometry analysis and the non-hematological malignancy is confirmed by being negative for CD45 and positive for TTF, Pan cytokeratin and cytokeratin [7].

According to the duration between the diagnosis of first and second malignancy, this is a case of metachronous second malignancy. It could be explained by the presence of a predisposing factor for the development of the first malignancy that makes the patient a higher risk candidate for a second de novo malignancy [5]. It is well known that metachronous malignancy occurs as a side effect of exposure to chemotherapy. In this report, the patient received azacytidine which is a cytosine analogue that functions as a DNA methyltransferase inhibitor, and is known to show effective potency in reactivating epigenetically silenced tumor suppressor genes, in vitro [9]. However, it has been difficult to define the mode of action in vivo, and it appears that clinical responses are affected by epigenetic alterations and induction of apoptosis [10]. In addition, the drug can induce epigenetic side effects, like the activation of silenced retroelements [11] that needs further studies of their effects and whether they do or do not induce second malignancy.

The incidence of multiple primary malignancy is variable among different studies. In a recently published four year study, in a geographically close Regional Cancer Institute, a metachronous malignancy incidence rate of 0.18% was recorded; a rate which is around four-fold greater than that for synchronous malignancy [12]. To the best of our knowledge, no reports from Saudi Arabia regarding the incidence of double malignancy exist and this is the first reported case to be published.

The incidence of development of solid tumor with a hematological malignancy is very rare. The implicated solid tumors reported are cancer in the esophagus, stomach, colon, and breast; with the major hematological malignancies reported being MM, MDS, NHL and CML [13]. Herein we report a case of initial AML followed by adenocarcinoma of the lung. According to a retrospective study published in 2014, lung cancer was the most common solid malignancy developed in patients with hematological malignancies [14].

This case was diagnosed in an elderly male with an unrelated positive family history. Being a patient above the age of 60 years old has been confirmed in other publications as increasing risk of malignancy, with most cases of double primary malignancy belonging to adults in the 5th to 6th age decades of life and beyond [4 &15]. Additionally, a Japanese study reported that elderly patients with hematologic malignancies are more likely to have multiple malignant neoplasms than younger patients [16]. Also, a predominance of malignancy was reported for males in the reviewed literature [6 &17].

In the current report, ten months after the patients' initial diagnosis, serum ferritin were noted to be elevated and continued to show a higher value till the diagnosis of the second malignancy. It is widely accepted that ferritin is an acute-phase reactant and can be nonspecifically elevated in a wide variety of inflammatory states including infection, malignancy, and autoimmune diseases [18]. These data should alert the attention of the clinician to consider the presence of a further clinical issue. Furthermore, the prolactin level was elevated which is highly suspicious of ectopic hormone production and should be considered as an early negative poor predictive factor for clinical outcome [19].

The elevated levels of the tumor markers; CA19-9 and CEA were highly suspicious for a second solid malignancy irrespective of its type. The CA 19-9 antigen is a glycoprotein synthesized in several epithelia that is typically high in the serum of patients with pancreatic tumors. Although uncommon, it is a fact that serum levels of CA 19-9 are increased in lung tumors, especially in adenocarcinomas [20 and 21]. The dual elevation of both CEA and CA19-9 has been reported in malignant lung lesions and provides a useful tool for diagnosis of lung cancer, especially if the tumor cannot be visualized by bronchofibroscopy [22]

In this case the second tumor presented in the form of metastasis in the BM denoting the advanced stage of the disease and the patient died two months after the diagnosis of the 2nd malignancy. The prognosis of double malignancy can be speculated based on the stage of each cancer, with previous reports indicating that prognosis would be good if the tumors were at early stage, otherwise the prognosis was likely poor [11].

6. Conclusion

With recent advances in diagnostic and staging modalities, as well as progress in the management of common cancer, the possibility of the existence of a second primary malignancies should always be considered. This is especially the case, whenever a novel laboratory finding emerges, that could be an early indicator in the detection of associated tumors, preferably before clinical manifestations occurrence. Generally, thorough regular follow up with proper result interpretation could detect most of the metachronous second primary malignancies at an early stage.

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