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CASE REPORT

Alveolar Proteinosis, Infectious Complications and Monocytopenia Associated with GATA2 Deficiency

Stefan Raffac[1], Mundher Abdulkareem Salman Aljubouri[2], Juliana Gabzdilova[3]

1 Department of Clinical Immunology, L. Pasteur University Hospital, Kosice, Slovakia
2 Department of Internal medicine, Medical faculty P.J. Safarik University and L. Pasteur University Hospital, Kosice, Slovakia
3 Department of Clinical Hematology and Onco-hematology, Medical Faculty P.J. Safarik University and L. Pasteur University Hospital, Kosice, Slovakia

Correspondence to: Juliana Gabzdilova, MD, PhD
Dep of clinical hematology and onco-hematology, Medical Faculty of P.J. Safarik University and L. Pasteur University Hospital, Kosice, Slovakia
tel.: +421903068259; e-mail: juliana.gabzdilova@unlp.sk

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Abstract

The authors describe a case of a rare disorder associated with GATA 2 deficiency, which is an important hematopoietic transcription factor for the development of monocytes. The clinical hallmarks of GATA2 deficiency include monocytopenia, cellular immunodeficiency with a resultant marked susceptibility to infections (mycobacterial, fungal, and viral), predisposition to myelodysplasia, alveolar proteinosis of the lungs and congenital lymphoedema. These features overlap with other genetic and acquired syndromes. It is therefore important to establish a genetic diagnosis early. The only known curative treatment is hematopoietic stem cell transplantation and is integral to the appropriate management of these patients.

Abbreviations:

GATA2	- GATA-binding factor 2
MRCP	- magnetic resonance cholangiopancreatography
USG	- ultrasonography
CT	- computed tomography
HRCT	- high resolution computed tomography
TBC	- tuberculosis
EBV	- Epstein-Barr virus
HIV	- Human immunodeficiency virus
HSV	- Herpes simplex virus
MDS	- myelodysplastic syndrome
AML	- acute myeloblastic leukemia
CRP	- C-reactive protein
PCT	- procalcitonin
DNA	- deoxyribonucleic acid
PCR	- polymerase chain reaction
ANA	- antineutrophil antibodies
ENA	- extractable nuclear antigen antibodies
ANCA	- anti-neutrophil cytoplasmic antibodies
CIK	- circulating immune complexes
CD	- cluster of differentiation
CS	- corticosteroids
FISH	- Fluorescence in situ hybridization
EBNA	- Epstein a-Barr nuclear antigen
WT1 gene	- Wilms's tumor 1 gene
IL	- Interleukin
HSC	- hematopoietic stem cells
HPV	- Human papilloma virus
INF-α	- Interferon α
PET CT	- positron emission tomographic scan
MonoMAC	- Monocytopenia and mycobacterial infection

INTRODUCTION

GATA2 deficiency, caused by a heterozygous mutation of the GATA2 gene, presents with a wide spectrum of clinical phenotypes. It is typically characterized by an increased susceptibility to infections (bacterial, mycobacterial, fungal, and viral), hematological abnormalities such as multi-lineage

cytopenia, myelodysplasia (MDS), aplastic anemia and leukemic transformation, as well as lymphedema and deafness (Emberger syndrome). Allogenic hematopoietic stem cell transplantation is considered to be the only curative therapy but is associated with significant treatment-related morbidity and mortality. Exact and early diagnosis is crucial to identify patients with this syndrome as the late stage of this disease is associated with increased mortality and severe complications that contraindicate bone marrow transplantation (Vinh *et al.* 2010).

CASE REPORT

A 54 year- old Caucasian male was admitted to the hospital due to a 3-weeks long fever of unknown origin, weakness, and weight loss (7kg in 18 days). Laboratory findings, abdominal ultrasound (USG) and Magnetic Resonance Cholangiopancreatography (MRCP) were indicative of acute cholangitis (hepatopathy and hypoechogenic lesions of the liver were demonstrated). While a chest CT demonstrated a nodular lesion of the lung. Acute respiratory insufficiency had developed a few days following admission, and this was attributed to the development of interstitial pneumonia (pneumonitis) identified on a high-resolution computed tomography (HRCT) scan of the chest (Figure 1).

Past medical history revealed a cholecystectomy due to cholecystitis at the age of 39 with protracted healing and long-term antibiotic therapy. At that time a mild thrombocytopenia and leukopenia (bi-cytopenia) were detected and these changes in blood count persisted for a long time (approximately 4 years according to available documentation) with no further investigation. At the age of 45 he was diagnosed with a protracted bilateral bronchopneumonia and was followed-up for chronic prostatitis as well.

A process of differential diagnosis of the pneumonitis was started. A detailed microbiological testing was done including cultivations of swabs from the throat and nose, as well as sputum, urine, and blood. The detection of HIV virus, HSV 1 and 2, EBV and CMV both serologically and by means of PCR was also attempted, alongside candida and aspergillus antigens, Pneumocystis jirovecii and tuberculosis (TBC). **Results demonstrated an elevation of Epstein-Barr virus DNA copies (PCR) in both serum and bronchoalveolar fluid (Table 1)**. HIV was negative and no fungal pathogens or TBC tested positive.

Fever and elevation of inflammatory parameters (C-reactive protein, Procalcitonin) continued despite the use of broad-spectrum antibiotics and antifungal therapy. Immunologists recommended the use of the immunomodulator drug Azoximer bromide as well as intravenous immunoglobulin substitution, but the fever persisted nonetheless.

A positron emission tomography scan (PET-CT) showed multiple, metabolically active lesions in the

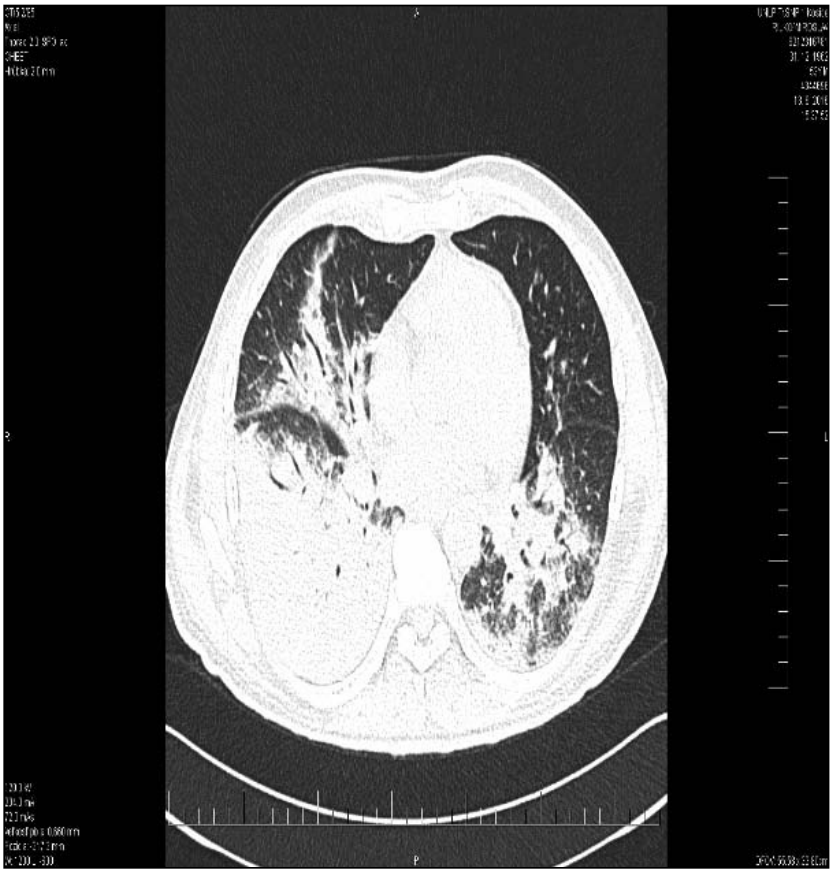

Fig. 1. CT scan of the lung. Both lungs show opacifications of a peri-bronchovascular distribution and interstitial thickening representing inflammatory infiltrates. Segments of the lower right lobe are mostly affected and form a condensation of pulmonary tissue and as well as a positive bronchogram. This finding is not specific for any etiology (bacterial, viral, mycotic, atypical interstitial). a Non-significant fluidothorax (10mm) is also present. Reactive enlargement of the lymphatic nodes is also present in the mediastinum – paratracheal, para-aortal, and subcarinal nodes with a maximal size of 12 mm. Splenomegaly is present as well.

Tab. 1. The viral load of EBV in specimens of the patient

Date	EBV copies /ml blood	EBV copies/ ml bronchoalveolar fluid
15.6.2016	-	358
11.9.2016	41 3000	-
29.9.2016	26 750	-

lungs, spleen, mediastinum and the bone marrow. Altogether, changes in peripheral blood count, mediastinal lymphadenopathy and splenomegaly led to the suspicion for a hematological disorder. Repeated flow cytometry examination of peripheral blood did not however, demonstrate any dysplasia or a hematological malignancy. But the **absence of monocytes** was a frequently dominant finding. Bone marrow biopsy did not demonstrate any dysplasia, lymphoproliferation or acute hemoblastosis.

Rheumatological screening revealed an elevation in serum circulating immunocomplexes, while antibody screening (ANA, ENA, ANCA) was negative. Complex immunological testing was also done and this included cellular immunity, humoral immunity (quantity of immunoglobulins, IgG subclasses detection and post-vaccination immunoglobulin titers). This revealed a **deficiency in cellular immunity** (CD4+ lymphopenia, deficiency in NK-cells and monocytopenia) (Table 2, Figure 2). These results led to the suspicion for autoimmune necrotizing vasculitis of the lungs and thus Corticosteroid (CS) therapy was started (pulses of methylprednisolone). This resulted in a decrease of inflammatory parameters and the remission of fever.

Six weeks following the first hospitalization, the patient was admitted to The National Center for lung diseases, where endobronchial sonography and transbronchial needle aspiration of lymph nodes was done to ascertain the diagnosis. This did not demonstrate any malignant changes, but the clinical respiratory status of the patient was getting worse. This led to treatment with another cycle of methylprednisolone and Cyclophosphamide. four weeks later, a severe pancytopenia and nephrotic proteinuria had manifested and there were signs of suspected autoimmune multiorgan damage. The bone marrow was also affected by the use of cyclophosphamide. A series of tissue histopathological examination was done. Bone marrow showed mild hypercellularity with no typical signs of dysplasia, but it did confirm the absence of monocytes mentioned earlier. Renal biopsy showed no signs of vasculitis, glomerulonephritis and immunofluorescence examination was negative. The most relevant findings were through pulmonary histological examination which demonstrated **alveolar proteinosis**, while EBV PCR serum positivity **persisted** and IgG EBNA was positive. The patient was treated with symptomatic therapy, antibiotics, and immunomodulatory agents.

Pneumothorax and acute respiratory insufficiency had ensued a few days following the lung biopsy. intensive care was therefore necessary where resuscitation and catecholamine infusion were started. Broad spectrum antibiotic therapy was continued in concomitance with intravenous immunoglobulins and Azoximer bromide. A follow-up CT scan of the chest demonstrated the progression of mediastinal lymphadenopathy and bilateral pneumonia with the development of an abscess on the right side. While changes typical for interstitial inflammation in the left lobes were observed as well as a residual pneumothorax in right ventro-apical and ventro-basal localizations.

Immunologists were repeatedly consulted throughout each hospitalization. They recommended supportive immunotherapy and proclaimed a suspicion for either MonoMAC syndrome, idiopathic CD4+ lymphopenia or X-linked lymphoproliferative syndrome. Genetic testing confirmed a GATA2 mutation. We elucidated **a variant that was not yet described and leads** to a Frameshift-type mutation producing a stop codon in the 16th position of the amino acid sequence of the GATA2 gene. Null variants (frameshift) affecting the GATA2 gene are a known

Tab. 2. Changes in lymphocyte numbers

Cellular immunity	–	6/2016	7/2016	9/2016	10/2016	11/2016
CD 3+	[10^9/l]	1,13	0,75	0,82	0,65*	0,64*
CD 4+	[10^9/l]	0,28*	0,22*	0,27*	0,20*	0,07*
CD 8+	[10^9/l]	0,76	0,5	0,5	0,42	0,52
CD 19+	[10^9/l]	0,1	0,10*	0,04*	0,1	0,32
NK cells	[10^9/l]	0,01*	0,01*	0,04*	0,02*	0,03*
CD 3+	[%]	89,00*	86,00*	87,00*	82	65
CD 4+	[%]	22,00*	25,00*	29	25,00*	7,00*
CD 8+	[%]	60,00*	58,00*	53,00*	53,00*	53,00*
CD 19+	[%]	8	11	4,00*	13	33,00*
NK cells	[%]	1,00*	1,00*	4,00*	3,00*	3,00*

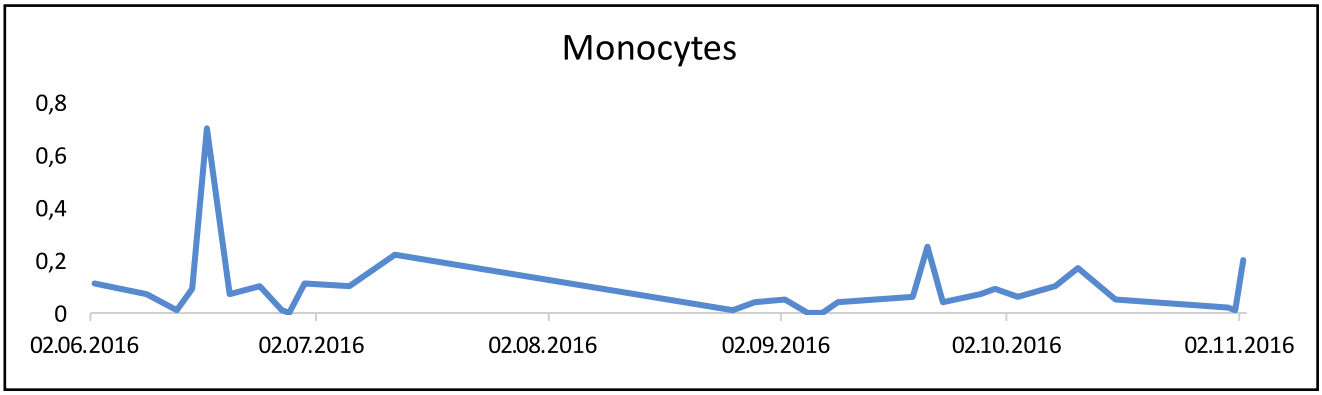

Fig. 2. Monocyte levels throughout a 5-month period.

mechanism of disease (26 pathogenic variants out of 109 classified variants = 23.85% which is greater than the threshold of 10.0%). Cytogenetics and fluorescence in-situ hybridization (FISH) testing implemented within the bone marrow examination revealed that 90% of the cells show a pathological karyotype of 47XY, with trisomy of the 8th chromosome, {47, XY, +8 (12)/46, XY, / (2)}. Molecular analysis of the bone marrow demonstrates overexpression of the WT1 gene transcript.

A search for macrophage-colony stimulating factor-medication, as well as IL-2 was done. Since monocytopenia without acute hemoblastosis was now confirmed. but these drugs were unattainable in our country. Unfortunately, the patient's clinical status was unfavorable and despite intensive therapy, had proven fatal. An autopsy was requested but was opposed by the family of the patient. Following the reception of the genetic results, genetic consultation was done for the family members.

DISCUSSION

The first case of GATA2 deficiency was published as a monocytopenia with susceptibility to non-tuberculous mycobacterial and atypical infections, as well as myelodysplasia and acute myeloblastic leukemia (AML). This condition was called the MonoMAC syndrome (monocytopenia and mycobacterial infection) and a GATA2 mutation was detected later. New clinical features of GATA2 mutations were frequently described. This was followed by the description of Emberger's syndrome, with primary lymphedema and AML, changes in subsets of lymphocytes, sensorineural deafness, different forms of EBV infections, and erythema nodosum with micro-organism-void granulomas. All these findings have shown that GATA2 is single genetic protean disease with a wide range of clinical hematological and non-hematological manifestations with a predisposition to hematological malignancies, mostly myelodysplastic syndrome (MDS) and consequently acute myeloblastic leukemia (AML) (Vinh *et al.* 2010; Ostergaard *et al.* 2011; Bodor *et al.* 2012; Johnson *et al.* 2015).

GATA1 and GATA2 are important factors involved in the process of hematopoiesis, with GATA1 being important for the development of red blood cells and platelets, and GATA2 for the development of hematopoietic stem cells and progenitor cells. GATA3 is important for the development of T cells. The human GATA2 gene is located on chromosome 3q21, has six exons, and belongs to a family of zinc finger transcription factors that are critical regulators of hematopoiesis. GATA2 transcription is regulated by several transcription factors (such as ETS1, BMP4, NOTCH1, PU.1, and EVI1) as well as the cytokines IL-1 and TNFα. The dynamic expression patterns of GATA1 and GATA2 critically influence the coordination of hematopoietic homeostasis, including genesis, survival, and maintenance of hematopoietic stem cells (HSC) and progenitor cells (Schimizu &Yamamoto 2016). down-regulation of GATA2 appears to be necessary for lineage differentiation.

In this case report we present a patient who developed bi-cytopenia (mild leukopenia and thrombocytopenia) that was the first sign of disease as a young adult (20-30y). These abnormalities were not accompanied by any dramatic changes for a protracted period. Later, Infections (pneumonia, chronic prostatitis) were observed in the medical history of our patient. At the age of 50 years the clinical status had become critical, with fever, pneumonitis and progressive lung injury being dominant. This was accompanied by a decrease in monocytes, NK cell, T and B lymphocytes. The course of the disease is consistent with the analysis of Spinner *et al.* (Spinner *et al.* 2014) and Donadieau *et al.* (2018). Where they analyzed 57 patients with GATA2 mutations. They described early symptoms in the form of common infections in the first decade of life, followed by a progression of infectious complications (bacterial, fungal, viral) and hematological complications in the second decade. Lung infections were dominant in up to 2/3 of patients. Changes in blood count were present even before the development of hematological disease where monocytopenia, neutropenia and thrombocytopenia were dominant. Regarding the immunological

parameters, approximately 50% of the patients had monocytopenia and B cell lymphopenia with normal immunoglobulin levels. The risk of severe complications increased with age, from severe infections, to the development of hematological malignant disease (81% risk at the age of 40 years) such as MDS, AML as well as T-lymphocyte acute lymphoblastic leukemia and juvenile myelo-monocytic leukemia. The suspicion for the development of hematological malignancy (MDS) was warranted in our patient, but repeated bone marrow examination did not demonstrate any pathological process in the bone marrow, only reactive changes associated with infections and the absence of monocytes.

Experience with EBV infection in GATA 2 deficiency was also reported by Cohen *et al.* (2016) in a group that consisted of 7 patients. In addition to having severe EBV disease, these patients had severe infections with herpes simplex virus, human papillomavirus, and nontuberculous mycobacteria. **The virus was detected in multiple tissue samples, including the bone marrow, lymph nodes, and spleen in patients without EBV-associated cancers.** While in patients with EBV-associated cancers (2 with smooth muscle tumors and 1 with Hydroa Vacciniforme–like lymphoma and T-cell lymphoma), the virus was detected in multiple tissues, including the liver, eyes, lungs, and small and large intestine. GATA2 deficiency is the first genetic disorder reported to be associated with EBV Hydroa Vacciniforme–like lymphoma. Cohen *et al.* concluded that EBV disease associated with GATA2 insufficiency was probably due to impaired immune surveillance against EBV due to reduced numbers and/or function of T and NK cells, rather than being an effect of GATA2 transcription in EBV-infected B cells. The median level of EBV DNA in the blood of EBV seropositive patients without EBV disease was 117 copies/ml, while the level was 14,750 copies/ml in patients with EBV disease. All of the reported patients had low numbers of monocytes, CD4 T, B, and NK cells. EBV infection was confirmed in our patient by detection of 358 EBV copies in bronchial fluid and persistence of the viremia was demonstrated by repeated positivity of EBV DNA copies in blood (41 300 and 26,500 copies).

Complex genetic analysis of the patient confirmed a deletion of the fifth nucleotide of the coding DNA strand, which causes the reading frame to shift from the second amino acid of the protein. This shift leads to the generation of a STOP codon at the position of the 16th amino acid and this terminates protein synthesis. Due to the nature of the mutation (null mutation) there is no doubt about the pathogenicity of this variant. The mutation was not found in any population database (neither in the database GnomAD, which has a larger population study than ExAC and is probably used more often, but ExAC was used as well in Donadieu *et al.* 2018 and it's not found there either). Karyotyping showed trisomy of the 8th chromosome and overexpression of the WT1 gene. Mutations in published analyzes were mainly located in exons 4 and 5. Four patients (8% of a cohort) had a complete heterozygous GATA2 locus deletion. **And 11 small deletions or insertions leading to predicted stop codons (21%).** There was a significant risk of developing leukemia in the group of patients with the missense mutations (14 of 38) versus the group with nonsense or frameshift mutations (2 of 28; P=0.007, Fisher's exact test). The majority of patients (>90%) **will present with a life-threatening hematologic and/or infectious manifestation by the age of 40. Karyotypes were abnormal in 43 of 66 patients (65%), with a complete or a partial loss of chromosome 7 in 27 cases (35%), trisomy-8 in 16 cases (18%) and 4 patients combining the two. The same karyotype with trisomy of 8th was confirmed in our case as well as overexpression in WT1 gene, that are associated with negative prognosis in patients with AML.** As previously published data suggest, the acquisition of additional genetic abnormalities in the transformation of GATA2 mutations to multilineage dysplasia is clearly presaged by the high incidence of monosomy 7 and trisomy 8 in familial cases of MDS/AML. Other secondary genetic events are probably responsible for the clinical variability among cases with GATA2 deficiency as well as the progression into myelodysplasia and acute leukemia as suggested by recently published papers and cases and these include EZH2, HECW2, and GATA1 (Donadieu *et al.* 2018). Other genes reported in different studies include *ASXL1* mutations, as well as mutations in the RAS pathway and AML/MDS mutated genes (Bödör at al. 2012; West *et al.* 2014; Wang *et al.* 2015).

Allogenic transplantation of hematopoietic stem cells is currently the only curative method for GATA2 associated MDS/AML and immune dysfunction. This treatment demonstrated efficacy in pulmonary alveolar proteinosis as well. The rationale for efficacy being via the reconstitution of the alveolar macrophages function (Mir 2015).

Transplantation can be especially challenging in this disease due to concurrent infections, pre-existing leukemia, or transformation of myelodysplastic syndrome into leukemia. Cases of disease relapse after hematopoietic stem cell transplantation and graft rejection suggest that myeloablative conditioning may be utilized, even though pre-existing comorbidities, including severe infections, may render myeloablation challenging in these patients. Antiviral therapy is generally ineffective for EBV disease associated with GATA2 deficiency. Treatment of some patients with GATA2 insufficiency with IFN – α resulted in increased numbers and/or function of NK cells, but did not increase the number of CD56 bright cells. Rituximab maybe effective in EBV-positive B cell tumors, but in the absence of reconstitution of the immune system, the disease can recur with CD20-negative B cell tumors. Treatment with third party EBV-specific T cells might provide temporary therapy prior to hematopoietic stem cell transplantation (Mir 2015).

CONCLUSION

This case illustrates the complicated course and problems encountered throughout the diagnosis and treatment of patients with GATA2 deficiency presenting with EBV infection, pulmonary proteinosis, monocytopenia and changes in cellular immunity. The disease course shows how severe are the conditions associated with cellular immunodeficiency and organ damage associated with GATA2 mutation and uncontrolled viral (EBV) infection. It also elucidated the presence of cytogenetic changes associated with high risk in patients with MDS/ AML and their role in potentiating a progression to hemoblastosis and myeloid dysplasia. This case should point out the importance of detailed monitoring and differentiation of leukopenia (monocytopenia) and searching for the above-mentioned conditions and close monitoring of these patients, especially the potential progression to hematological malignancy. The only curative method in this situation is allogenic bone marrow transplantation. which also positively influences end-organ damage (e.g. pulmonary proteinosis). This case may help in guiding the diagnostic and treatment decisions of other patients with similar clinical presentation.

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