Patients with acute promyelocytic leukemia (APL) often present with potentially life-threatening hemorrhagic diathesis. The underlying pathomechanisms of APL-associated coagulopathy are complex. However, two pathways considered to be APL-specific had been identified: 1) annexin A2 (ANXA2)-associated hyperfibrinolysis and 2) podoplanin (PDPN)-mediated platelet activation and aggregation. In contrast, since disseminated intravascular coagulation (DIC) is far less frequent in patients with non-APL acute myeloid leukemia (AML), the pathophysiology of AML-associated hemorrhagic disorders is not well understood. Furthermore, the potential threat of coagulopathy in non-APL AML patients may be underestimated. Herein, we report a patient with non-APL AML presenting with severe coagulopathy with hyperfibrinolysis. Since his clinical course resembled a prototypical APL-associated hemorrhagic disorder, we hypothesized pathophysiological similarities. Performing multiparametric flow cytometry (MFC) and immunofluorescence imaging (IF) studies, we found the patient’s bone-marrow mononuclear cells (BM-MNC) to express ANXA2 - a biomarker previously thought to be APL-specific. In addition, whole-exome sequencing (WES) on sorted BM-MNC (leukemia-associated immunophenotype (LAIP)1: ANXAlo, LAIP2: ANXAh) demonstrated high intratumor heterogeneity. Since ANXA2 regulation is not well understood, further research to determine the coagulopathy-initiating events in AML and APL is indicated. Moreover, ANXA2 and PDPN MFC assessment as a tool to determine the risk of life-threatening DIC in AML and APL patients should be evaluated.

Keywords: acute promyelocytic leukemia, acute myeloid leukemia, hyperfibrinolysis, disseminated intravascular coagulation, coagulopathy, ANXA2, PDPN, WES
INTRODUCTION

Patients with APL often present with potentially life-threatening hemorrhagic diathesis (1). Here, pathophysiology of APL-associated coagulopathy is complex (2). Beside enhanced thrombin activation inducing disseminated intravascular coagulation (DIC), annexin A2 (ANXA2) mediated hyperfibrinolysis has been identified as a key-pathway (3). ANXA2, encoded by the ANXA2 gene located on chromosome 15, serves as a cell surface receptor for both, plasminogen (PLG) and tissue-type plasminogen activator (PLAT), accelerating plasin formation (4). Thus, ANXA2 overexpression on APL promyelocytes is thought to cause hyperfibrinolysis (5).

In addition to the well-known mediators of APL-associated coagulopathy, such as tissue factor, ANXA2 and PLAT, podoplanin (PDPN) - previously known as a marker of coagulopathy, such as tissue factor, ANXA2 and PLAT, podoplanin (PDPN) - previously known as a marker of

In contrast, coagulopathy/hyperfibrinolysis is less frequent in patients with non-APL AML. Therefore, 1) the potential threat of coagulopathy in non-APL AML patients may be underestimated and 2) underlying pathophysiology of AML-associated hemorrhagic disorders is not well understood.

CASE DESCRIPTION

A 64-year-old man (unique patient number (UPN) 1) noticed spontaneous bruising of the arms and legs about six weeks before admission, at the same time progressive fatigue and night sweats developed. Due to episodes of spontaneous, prolonged gingival hemorrhage the patient presented to his primary care doctor two weeks later, whereupon the patient was referred to our center. On admission, the patient’s white blood cell (WBC) count was 5.3 x 10^9/L (normal: 3.8-9.8 x 10^9/L), hemoglobin was 4.65 mmol/L (normal: 8.6-12.1 mmol/L), and platelet count was 51,000 x 10^9/L (normal 150,000-400,000 x 10^9/L) with a WBC differential including 27% neutrophils, 25% monocytes, 38% lymphocytes, 1% eosinophils, and 9% myeloblasts. Prothrombin ratio (PR) was 49% (normal: 70-120%), activated partial thromboplastin time (aPTT) 40 s (normal: 24-36 s), initial fibrinogen level 1.28 g/L (normal: 2-4 g/L), D-dimer level >4 µg/mL FEU (normal: <0.5 µg/mL FEU), PR 39% (normal: 70-120%), aPTT 36 s (normal: 24-36 s), antithrombin activity 99% (normal: 80-120%), and ISTH DIC score was 8 (normal: <5), resembling the situation at initial presentation (Figure 1). Once more extensive supplementation of platelets, fibrinogen and tranexamic acid was required to control ongoing coagulopathy. Simultaneously, a sequential conditioning regimen according FLAMSA-RIC (fludarabine, amsacrine and cytarabine followed by fludarabine, busulfan and anti-thymocyte globulin) was performed since the patient did not achieve complete remission. On day 70 following initial presentation the patient underwent peripheral blood HCT from a matched unrelated donor with HLA-DPB1 non-permissive mismatch. Cyclosporine A and mycophenolate mofetil were administered as graft-versus-host disease (GvHD) prophylaxis. Beyond grade 3 mucositis and neutropenic fever, further aplasia took a complication-free course and platelet and neutrophil engraftment occurred on day +14 and +18 following HCT, respectively.

Two months after transplantation, examination of a bone marrow–biopsy specimen revealed complete morphologic and molecular remission and a full donor chimerism. On last follow-up, 12 months after transplantation, the patient is free of complaints and remains in complete remission.

METHODS

We determined ANXA2 and PLAT expression on the patient’s (UPN1) bone marrow derived mononucleated cells (BM-MNCs) via multiparametric flow cytometry (MFC) and immunofluorescence staining of cytospin samples (IF). Furthermore, PDPN expression was examined via IF. Further, whole exome sequencing (WES) was performed on sorted UPN1 BM-MNCs populations: Briefly, DNA - isolated from BM-MNC
populations - was quantified using the Qubit dsDNA HS Assay (Q32851, Life Technologies). Library construction was performed from isolated DNA using TruSeq DNA Nano Sample Preparation kits (Illumina, San Diego, California, USA) according to the manufacturer’s instructions and indexed libraries were paired end (2x151 bp) sequenced on Illumina HiSeqX instrument (Illumina). FASTQ files were generated using the BCL2 fastQ pipeline (bcl2fastq 2.19.0.316). Per sample, on average 498M (range 455M-514M) read ends were obtained. HG19 was used as reference genome for bioinformatic analyses. The bioinformatics evaluation was performed using the Biomedical Workbench from CLC (12.0.3) using a customized analysis algorithm with following filters: coverage >=25, variant allele frequency >=10%. SNVs were further annotated for their biological effect and filtered based on SNV-quality, minimum number of supporting reads and biological relevance (non-synonymous SNVs). Variant assessment/classification was performed using VarSome, which includes—among others—ClinVar and dbSNP databases, population frequency information from gnomAD and in-silico prediction tools such as FATHMM, SIFT, REVEL and Polyphen2 (10). Only variants classified as pathogenic, likely pathogenic or variants of uncertain significance (VUS) - according to the American College of Medical Genetics and Genomics (ACMG) - were reported (11). BM-MNCs obtained from a patient with APL (UPN 2), a patient diagnosed with AML (FAB M2) with KMT2A-PTD and an IDH2 mutation without evidence of DIC (UPN3), and a healthy donor served as positive and negative control, respectively. Here, BM-MNCs were obtained from the Study Alliance Leukemia (SAL) biobank, which has been...
approved by the Ethics Committee of the TU Dresden (EK98032010). Written informed consent had been obtained from all participants.

RESULTS AND DISCUSSION

MFC revealed pronounced ANXA2 expression on patients BM-MNCs (SSC<lop/mid>CD45<dim>) (Figure 2A). Furthermore, MFC identified two leukemia-associated immunophenotypes (LAIP) with low (LAIP1: SSC<lop/mid>CD45<dim>CD13<sup>+</sup>CD33<sup>+</sup>HLA-DR<sup>+</sup>CD56<sup>-</sup>CD117<sup>+</sup>) and high (LAIP2: SSC<lop/mid>CD45<dim>CD13<sup>-</sup>CD33<sup>+</sup>HLA-DR<sup>+</sup>CD56<sup>-</sup>CD117<sup>+</sup>) ANXA2 expression, respectively (Figure 2A). Notably, via MFC no ANXA2 expression was seen on healthy donor BM-MNCs (SSC<lop/mid>CD45<dim>) (data not shown).

IF confirmed marked ANXA2 expression on patients BM-MNCs and demonstrated distinct ANXA2/PLAT co-expression, resembling UPN2 (APL) BM-MNCs phenotype (Figure 2B). In contrast, PDPN expression was exclusively seen on UPN2 BM-MNCs. BM-MNCs obtained from UPN3 (AML with maturation; FAB M2) neither showed ANXA2 nor PDPN expression, only low-level PLAT expression was observed. Scale bar corresponds to 20µm.

FIGURE 2 | Assessment of patients BM-MNCs via MFC, IF and WES. (A) Assessment of ANXA2 in UPN1 BM-MNCs populations via MFC: BM-MNCs (SSC<lop/mid>CD45<dim>) [red] showed distinct ANXA2 expression on initial presentation as well as pre alloHCT. On closer examination, 2 LAIP were identified: LAIP1 (SSC<lop/mid>CD45<dim>CD13<sup>+</sup>CD33<sup>+</sup>HLA-DR<sup>+</sup>CD56<sup>-</sup>CD117<sup>+</sup>) [blue] showed nearly no ANXA2 expression, whereas LAIP2 (SSC<lop/mid>CD45<dim>CD13<sup>-</sup>CD33<sup>+</sup>HLA-DR<sup>+</sup>CD56<sup>-</sup>CD117<sup>+</sup>) showed excessive ANXA2 expression [purple]. FMO: negative control [grey]. (B) Assessment of ANXA2, PLAT and PDPN via IF: BM-MNCs from UPN1 showed marked ANXA2 and PLAT expression, whereas no PDPN expression was observed. BM-MNCs from UPN2 (a patient diagnosed with APL) expressed ANXA2, PLAT as well as PDPN, whereas BM-MNCs from UPN3 (a patient diagnosed with AML [FAB M2]) with KMT2A-PTD and an IDH2 mutation without evidence of DIC) neither showed ANXA2 nor PDPN expression, only low-level PLAT expression was observed. Scale bar corresponds to 20µm. (C) Mutational profile of LAIP<sup>1</sup> (MFC ANXA2<sup>lo</sup>) and LAIP<sup>2</sup> (MFC ANXA2<sup>hi</sup>) as assessed by WES; T-lymphocytes served as a germline-like control. Columns represent LAIPs, rows represent genes and box color indicates the type of genomic alteration. Variants in bold print are exclusively found in LAIP2. Asterisk indicates previously known variants in IDH2 and SRSF2, allogeneic hematopoietic cell transplantation; ANXA2, annexin A2; AU, arbitrary units; BM-MNCs, bone marrow derived mononucleated cells; DIC, disseminated intravascular coagulation; FMO, fluorescence minus-one; LAIP, leukemia-associated immunophenotype; MFC, multiparametric flow cytometry; PLAT, tissue-type plasminogen activator; PDPN, podoplanin; UPN, unique patient number; WES, whole-exome sequencing.
evidence of KMT2A-PTD and IDH2 mutation; no evidence of DIC) showed neither ANXA2 nor PDNP expression, arguing against a correlation between evidence of KMT2A-PTD and/or IDH2 mutation and presence of DIC. In contrast, as a possible genotype/phenotype association, DiNardo et al. reported two patients diagnosed with AML with evidence of t(10;11)(p12;q23) translocation (KMT2A-MLLT10 rearrangement) presenting with DIC (12). However, 1) these patients presented with KMT2A-MLLT10 fusions, but not KMT2A-PTD and 2) in larger studies DIC was not reported as frequent event in adult patients diagnosed with t(v;11q23.3); DIC was not reported as frequent event in adult patients diagnosed with AML with evidence of t(10;11)(p12;q23) genotype/phenotype association, DiNardo et al. reported two mutations and presence of DIC. In contrast, as a possible fusions, but not

shown neither ANXA2 nor PDPN expression, arguing against a

expression and associated coagulopathy appears to be speci

can also occur in other AML subtypes. In contrast, PDPN

hyper

architecture, genomic characterization could not provide a valid link
to the severe coagulopathy with hyper

mechanosensitive ion channel component 1 (PIEZO1) to be associated with adverse prognosis in AML and piezo type

expression

 backgrounds and upstream regulation of ANXA2 and PLAT

To conclude, further research to 1) evaluate ANXA2 MFC assessment as a tool to determine the risk of life-threatening DIC in AML patients, 2) investigate PDNP as a marker of APL/APL-associated coagulopathy, 3) determine the efficacy and safety of anti fibrinolytic agents in preventing hemorrhage in people with hematological malignancies, and 4) understand the genetic background and upstream regulation of ANXA2 and PLAT and coagulopathy-initiation in non-APL AML and APL clones, is indicated.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because ethics restrictions apply. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by TU Dresden ethics committee (EK98032010). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LR, FS, and MB conceived the presented idea. LR, FS, LW, and MvB performed research. HA provided BM-MNC. AR and ES helped to perform molecular analysis. SH helped to assess WES data. LR wrote the manuscript with help from FS, UP, SH, JS, MB, and MvB. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.666014/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.