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Identifier: O18

DOMINANT NEGATIVE ADA2 MUTATIONS CAUSE ADA2 DEFICIENCY IN HETEROZYGOUS CARRIERS

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Introduction: Human ADA2 deficiency (DADA2) is an inborn error of immunity with a broad clinical phenotype encompassing vasculopathy including livedo racemosa and lacunar strokes as well as hemato-immunological manifestations. Combination of decreased serum ADA2 activity and the identification of biallelic deleterious alleles in the *ADA2* gene are used for diagnosis. DADA2 carriers harbor a single pathogenic variant in *ADA2* and are mostly considered healthy and asymptomatic. However, some DADA2 carriers present a phenotype compatible with DADA2 (1–4).

Objectives: We sought to investigate whether being heterozygote for specific variants in *ADA2* could explain the patients' DADA2 phenotype.

Methods: A HEK293T cell overexpression system was used to evaluate impact of ADA2 variants on WT ADA2 protein expression/secretion and enzymatic activity. Finngen, UK biobank and the Bio*Me* Biobank were used to assess population genetics and evaluate correlation with DADA2 phenotypes. ADA2 enzyme activity was measured in a colorimetric assay adapted from Giusti et al (5).

Results: In addition to diseased DADA2 carriers in literature (1–4), we report and investigate a cohort of 10 heterozygous carriers of pathogenic *ADA2* variants presenting with DADA2 clinical features. To study the potential effect of heterozygous pathogenic variants in ADA2 on WT ADA2 protein expression, secretion and enzymatic activity, we performed transient transfection of each *ADA2* variant together with WT *ADA2* to mimic carrier status. In vitro study of the *ADA2* variants identified in this patient cohort revealed that R169Q, H424N and Y453C variants affect secretion of WT ADA2 protein. Moreover, we demonstrate a dominant negative effect on the enzymatic activity of WT ADA2 by variants G47A, G47R, G47V, R169Q, E328K, H424N and Y453C both intracellularly and extracellularly. Data from PheWAS show that the heterozygous state for pLOF variants in *ADA2* is associated with phenotypes that align with DADA2. When studying the most frequent allele, R169Q, the enriched phenotypes are even more striking, despite the overall low number of cases.

Conclusion: Here, we describe how specific heterozygous variants cause ADA2 deficiency through distinct dominant negative effects on either ADA2 enzyme activity, dimerization and/or secretion. At the protein level, heterozygosity for these variants mimics what is observed in DADA2. We conclude that humans with heterozygous dominant negative missense variants in *ADA2* are at risk of DADA2.

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Disclosure of Interest: None declared

Identifier: O43

TARGETING THE DYSREGULATED TYPE I IFN RESPONSE IN ADENOSINE DEAMINASE 2 DEFICIENCY EFFECTIVELY MITIGATES INFLAMMATION VIA PATHWAY INHIBITION AND GENE THERAPY

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Introduction: Deficiency of Adenosine Deaminase 2 (DADA2) is a rare autoinflammatory disorder characterized by systemic inflammatory manifestations, including vasculopathy, cytopenias, and recurrent fevers. A hallmark of DADA2 is the heightened type I interferon (IFN) signature, driven by elevated IFN-β and interferon-stimulated gene (ISG) expression, which contributes to its pathogenesis. Understanding the mechanisms behind this hyperinflammatory state is critical for developing targeted therapies.

Objectives: This study investigated the mechanisms responsible for the increased type I IFN signature in DADA2, evaluated the therapeutic potential of targeting the cGAS-STING and JAK-STAT pathways, and explored gene therapy as a curative strategy to correct the inflammatory phenotype in DADA2.

Methods: Peripheral blood mononuclear cells (PBMCs) from DADA2 patients were analyzed for type I IFN and ISG expression. A human monocytic U937 cell line with ADA2 knockout (KO), generated using CRISPR-Cas9, served as an *in vitro* disease model. Pharmacological inhibitors of the cGAS-STING and JAK-STAT pathways were employed to modulate IFN signaling, and a zebrafish model of DADA2 was used to validate findings *in vivo*. Lentiviral transduction of ADA2-deficient macrophages with a vector encoding ADA2 assessed the potential of gene therapy in restoring normal IFN responses.

Results: PBMCs from DADA2 patients exhibited a markedly elevated type I IFN signature, with increased expression of IFN-β and ISGs, such as *ISG15*, *IFIT1*, and *RSAD2*. In ADA2 KO U937 cells, stimulation with the synthetic dsDNA analogue poly(dA:dT) recapitulated the heightened IFN response. Inhibition of the cGAS-STING pathway with Ru521 or H151 significantly reduced IFN-β levels and normalized the IFN response. Similarly, blocking the JAK-STAT pathway using ruxolitinib or an anti-IFNAR antibody restored IFN-β and ISG expression to baseline levels. The zebrafish model confirmed the heightened type I IFN response observed in human studies, and pharmacological inhibitors effectively mitigated the inflammatory response *in vivo*, supporting these pathways as viable therapeutic targets. Finally, gene therapy restored ADA2 expression in ADA2-deficient macrophages and normalized the type I IFN response. Lentiviral transduction fully reversed the hyperactive IFN response, demonstrating the potential of gene therapy as a curative approach for DADA2.

Conclusion: Our study identifies the cGAS-STING and JAK-STAT pathways as central drivers of the heightened type I IFN response in DADA2, providing strong evidence for pharmacological inhibition as a viable strategy to mitigate inflammation. Additionally, the successful restoration of ADA2 function and normalisation of the IFN response through gene therapy underscores its potential as a long-term curative strategy. These findings offer a roadmap for both immediate and future therapeutic interventions aimed at reducing the inflammatory burden in DADA2 patients.

Disclosure of Interest: None declared

Identifier: PT12

HUMAN ADA2 DEFICIENCY IS CHARACTERIZED BY THE ABSENCE OF AN INTRACELLULAR HYPOGLYCOSYLATED FORM OF ADENOSINE DEAMINASE 2

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Introduction: Human deficiency of adenosine deaminase 2 (DADA2) is a rare autoinflammatory disease with a complex clinical phenotype of recurrent fever, vasculitis and stroke as well as immunodeficiency and bone marrow failure. It is caused by pathogenic variants in *ADA2* that lead to impaired ADA2 protein secretion and reduced deaminase activity. Next to its role in the regulation of extracellular adenosine levels, ADA2 has recently been shown to mediate lysosomal nucleic acid sensing. However, ADA2 has primarily been established as a secretory protein and an intracellular form has not yet been characterized.

Objectives: The aim of this study was to analyze the processing and trafficking of wild-type (WT) and mutant ADA2, and thereby explore the cellular mechanisms driving DADA2.

Methods: We differentiated human monocyte-derived macrophages (HMDM) from 12 healthy controls (HC) and 10 DADA2 patients. Eleven pathogenic *ADA2* variants were overexpressed in HEK293T cells or transduced into *ADA2*$^{-/-}$ U-937 cells. ADA2 protein expression and molecular weight in these cells were assessed by western blot after sequentially inhibiting enzymes involved in N-glycan processing in the endoplasmic reticulum (ER), Golgi apparatus and lysosome or protein trafficking via the secretory pathway. Glycan removal was performed by PNGase F, Endo H and alpha-mannosidase. Localisation of ADA2 glycoforms was determined by subcellular fractionation.

Results: We identified a low-molecular-weight (LMW) form of ADA2 expressed exclusively intracellularly in HC HMDM and cell lines expressing WT ADA2. This LMW-ADA2 was subjected to glycan trimming by alpha-mannosidases after transfer to the Golgi and was distinct from secreted high-molecular-weight (HMW) ADA2. Cells expressing pathogenic *ADA2* variants including DADA2 patients' HMDM lacked LMW-ADA2. Mutant ADA2 was retained in the ER and did not undergo glycan processing in the Golgi apparatus. We confirmed the absence of LMW-ADA2 upon overexpression of pathogenic *ADA2* variants in HEK293T cells and monocytic U-937 cells as a feature shared by all examined DADA2-associated variants. By subcellular fractionation, we further showed that LMW-ADA2 localizes to the lysosomal compartment.

Conclusion: We describe a previously unreported intracellular hypoglycosylated form of ADA2 and establish the absence of this LMW-ADA2 as a cellular characteristic of DADA2. Thereby, we introduce a protein correlate of the recently described lysosomal form of ADA2 and highlight its absence in ADA2-deficient cells.

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Disclosure of Interest: None declared

Identifier: PT18

ASSESSMENT OF ADA2 ACTIVITY LEVELS: REPORT FROM THE ITALIAN STUDY GROUP ON DADA2

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Introduction: Adenosine Deaminase 2 deficiency (DADA2) is a rare monogenic autoinflammatory disease resulting from loss-of-function mutations in ADA2. Functional assays are crucial for early diagnosis. In 2021, we introduced a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to assess ADA2 activity from dried plasma spot (DPS).
Objectives: To define cut-offs of ADA2 activity in the normal population and to assess the test's utility in a large multicentre real-life cohort of patients with suspected DADA2.
Methods: In this prospective study, we included all patients who underwent the ADA2 functional assay by LC-MS/MS as a preliminary screening test in presence of a clinical picture potentially consistent with the diagnosis of DADA2 (group 1) or as a confirmatory functional test in subjects with previously detected biallelic or heterozygous ADA2 mutations (group 2).

We used the preliminary cut-off values for ADA2 enzymatic activity provided by our previous pilot study [1].

Moreover, to better define the cut-off levels, the enzymatic test was performed in healthy donors.

Receiver Operating Curves (ROC) analysis evaluated the diagnostic performance of ADA2 activity in DPS. Spearman correlation coefficients were employed to investigate the relations between ADA2 activity in DPS and age. Significance was determined at a threshold of $P<0.05$ for all analyses, with two-tailed tests utilized.

Results: A total of 219 symptomatic subjects were enrolled: 198 in group 1 and 21 in group 2.

5 subjects in group 1 exhibited a pathological ADA2 activity: all of them were confirmed to have biallelic pathogenic mutations in the *ADA2* gene. 6 patients had intermediate activity levels: 1 was found to have biallelic variants in the *ADA2* gene, while 5 did not have any mutations.

In 11 out of 15 patients of group 2 with biallelic ADA2 mutations, 11 presented a pathological ADA2 activity, 3 an intermediate activity wihile in 1 patient the activity was normal. Among 6 patients with heterozygous mutations, ADA2 activity was intermediate in 4 and normal in 2.

To better define the cut-off levels of ADA2 enzymatic activity we considered the data of the 219 patients included in this study, 17 previously reported patients with biallelic *ADA2* mutations [1], 22 asymptomatic carriers and 133 healthy donors.

The enzymatic test effectively discriminated between patients with biallelic *ADA2* mutations and carriers (AUC=0.951, $P<0.001$), between carriers and healthy donors (AUC=0.888, $P<0.001$), and between subjects with biallelic *ADA2* mutations and healthy donors (AUC=0.993, $P<0.001$), with high sensitivity and specificity.

Between the 133 healthy donors, a significant inverse correlation was found between ADA2 activity in DPS and age ($P<0.0001$).

The ADA2 activity cut-off values in DPS were identified as follows: ≤ 0.09 mU/mL for patients with DADA2, 0.10 – 0.39 mU/mL for carriers, and ≥ 0.40 mU/mL for subjects with normal levels. All patients with two pathogenic variants in the ADA2 gene exhibited ADA2 activity ≤ 0.09 mU/mL. For three patients, with a mild phenotype, exhibiting a pathogenic variant along with a VUS in the ADA2 gene, carrier-like ADA2 activity was observed, while 1 patient, carrying two VUS, the ADA2 activity was normal.

An overlap in ADA2 activity levels was observed between carriers and healthy subjects.

Conclusion: The LC-MS/MS ADA2 enzymatic test from DPS demonstrated to be easy to perform, quick and with high sensibility and specificity.

The presence of a pathological enzymatic activity should guide to further genetic tests, in case of a non-confirmatory genotype, while in patients with a non-confirmatory genotype a normal ADA2 activity can be of help to rule out the diagnosis of DADA2.

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Disclosure of Interest: None declared

Identifier: PT43

USING A T-CELL DIRECTED APPROACH IN THE TREATMENT OF DADA2-RELATED NEUTROPENIA RESULTS IN RECOVERY OF MYELOID CELL DEVELOPMENT PRE-TRANSPLANT AND SUCCESSFUL ENGRAFTMENT POST-TRANSPLANT

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Introduction: Deficiency of adenosine deaminase 2 (DADA2) is a genetic disease initially described with fevers, medium vessel vasculitis and recurrent, lacunar, ischemic strokes in children. Over the past decade the phenotype expanded to involve hematologic features such as various cytopenias including profound neutropenia. Administration of tumor necrosis factor inhibitors results in dramatic reduction in risk for ischemic strokes and decreased inflammatory burden of disease but little to no effect on neutropenia. Additionally, there have been multiple reports of graft failure in DADA2 patients undergoing transplant with neutropenia being their transplant indication.

Objectives: To better characterize and treat DADA2 patients with neutropenia.

Methods: Two patients with profound neutropenia underwent bone marrow biopsies to analyze cellular makeup. Post biopsy, cyclosporine was loaded at 5mg/kg/day for 6 days as well as a glucocorticoid taper of prednisone 30mg daily x 2days, 20mg x 2days, 10mg x 2days and then 5mg daily. Maintenance cyclosporine was approximately 2.5-3.5 mg/kg/day with levels followed closely.

Results: Patient 1 (R169Q/deletion in exon 7) is a 57 yo female with a 28 year history of neutropenia as well as arthalgias, rash, splenomegaly requiring splenectomy and fevers. Bone marrow biopsies completed in 1996 and 2023 revealed normal to hypercellular marrow (40-70%) with markedly decreased granulocytic precursors and extensive lymphocytic infiltrates and aggregates with antibody staining against CD3 identifying the majority of lymphocytes as T-cells. After DADA2 diagnosis, the patient was initially treated with subcutaneous immune globulin x 4 doses without improvement in neutropenia. Subsequently, she was initiated on cyclosporine and glucocorticoids with increasing ANC in 3 days from 200 to 800. She then was loaded with infliximab prior to switching to adalimumab. Within one week of cyclosporine initiation, ANC increased to 1600. Follow-up bone marrow biopsy performed 4 months after cyclosporine initiation showed normocellular marrow with progressive trilineage hematopoiesis and normal maturation of myeloid cells. Decreased, yet still present, T-cell lymphocytosis and multiple small T-cell lymphoid aggregates were observed. Five months post-cyclosporine initiation, ANC was 4560.

Patient 2 (R169Q/G47W) is a 17 yo male with a 2 year history of neutropenia and necrotizing, neutrophil rich, subcutaneous arteritis. At the time of DADA2 diagnosis, etanercept was initiated. Due to lack of hematologic response, he transferred to adalimumab. Despite resolution in skin rash and fevers, ANC remained 0. Bone marrow biopsy revealed normocellular marrow with a paucity of myeloid cells and significant lymphoid infiltration and lymphoid aggregates that were CD3 antibody positive for T-cells. One week after initiation of cyclosporine, ANC rose to 2900. The patient was maintained on this regimen for 9 months preceding transplant. Conditioning for transplant was T-cell directed including 3 days of pulse methylprednisolone and fludarabine. The patient had an uncomplicated post-transplant course with successful engraftment and remains disease free 5 years post-transplant.

Conclusion: DADA2-associated neutropenia has a strong T-cell infiltrate presented here on patient bone marrow biopsies. Expanding the treatment regimen in neutropenic DADA2 patients to include T-cell directed therapies has normalized ANCs in the pre-transplant period and sustained post-transplant engraftment. Notably, the infiltrate is less, albeit present, after adding the cyclosporine yet myeloid cells have become apparent. Additional studies utilizing alternative T-cell directed regimens are needed to further investigate this approach both for patients preparing for transplant and for those who are not transplant candidates.

Disclosure of Interest: None declared

Identifier: PT65

UNRAVELING GENETIC COMPLEXITY: DIFFERENT DISEASES IN SIBLINGS WITH SHARED CLINICAL PRESENTATION

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Introduction: Adenosine deaminase 2 (DADA2) deficiency and laccase domain-containing 1 (LACC1) deficiency are both rare autosomal recessive diseases with overlapping clinical presentations, including fever, arthritis, rash, and elevated inflammatory markers. DADA2 is caused by loss-of-function mutations in the ADA2 gene, which encodes the adenosine deaminase 2 (ADA2) protein. The clinical spectrum of DADA2 is broad, including vasculitis and autoinflammatory manifestations. Homozygous loss-of-expression mutations in LACC1 are associated with early onset arthritis.
Objectives: We identify a novel homozygous mutation in the LACC1 gene as the genetic cause of a disease in a sibling of a DADA2 patient who had similar symptoms.
Methods: We conducted a chart review of clinical and laboratory data. We performed whole genome sequencing (WGS) on the patient and functional validation to confirm the pathogenicity of the identified variant
Results: We describe a 9-year-old girl who presented with persistent fever, arthritis, rash, and elevated inflammatory markers (Table 1). Her sister, 18 years old, has DADA2 deficiency due to a homozygous *ADA2* pathogenic mutation (c.754-2A>G). This was discovered when she was 13 years old, presenting with fever, arthritis, and rash, to which she responded well to anti-TNF therapy (Table 1). Surprisingly, targeted Sanger sequencing for our patient revealed a heterozygous ADA2 mutation, similar to her consanguineous parents. The fact that the patient didn't improve with anti-TNF therapy, which is usually effective for DADA2, further raised suspicion of an alternative diagnosis. *Consequently, we performed WGS for our patient and discovered a novel homozygous variant of uncertain significance in the LACC1 gene.* Functional studies performed on monocyte-derived macrophages from the patient confirmed the loss of LACC1 expression (Figure 1). Thus, the patient was treated with IL-6 blockade (tocilizumab), and achieved an excellent clinical response, resolving her fever, rash, and arthritis while also normalizing her inflammatory markers.
Conclusion: We identified a novel pathogenic variant in the *LACC1* gene in a patient whose symptoms overlapped with those of her sister, who has a mutation in the *ADA2* gene. This case highlights the important role of genetic and functional validation in providing an accurate diagnosis and guiding personalized treatment for rare autoinflammatory diseases with overlapping clinical presentations.
Acknowledgments: Patients and family
Disclosure of Interest: None declared

Identifier: PT73

DISEASE PRESENTATION, RESPONSE TO TREATMENT AND OUTCOME OF PEDIATRIC AND ADULT PATIENTS WITH DADA2 (DEFICIENCY OF ADENOSINE DEAMINASE 2): RESULTS FROM THE EUROFEVER REGISTRY

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Introduction: DADA2 is a monogenic autoinflammatory condition characterised by a broad spectrum of clinical manifestation, ranging from cutaneous to severe systemic vasculitis with multiorgan involvement, immunodeficiency and
bone marrow failure. Few data are nowadays available about the clinical characteristic, the response to treatment and the outcome of this disease.
Objectives: To analyse the data of the DADA2 patients enrolled in the Eurofever registry.
Methods: The data analysed were extracted from the Eurofever registry, which is hosted in the PRINTO website. The patients were included in the study in the presence of clinical manifestations consistent with DADA2, a confirmatory genotype or a pathologic ADA2 enzymatic activity. Demographic data, clinical manifestations, treatment, safety and outcome were analysed.
Results: In May 2024 baseline and clinical information were available of 92 DADA2 patients (43M:49F), from 23 centers, in the Eurofever registry; of these, follow-up data were available for 63 patients (mean follow-up duration 2.8 years). 71 patients (77%) had a confirmatory genotype, while 21 a non-confirmatory genotype. 17 patients had a positive family history (12 couples of siblings were included in the registry). The mean age at enrolment was 12.5 years (74 paediatric and 18 adult patients), at disease onset 7,4 years (SD 8,6) and at diagnosis 15.2 years (SD 12), with a mean delay of 8 years.

The disease course was continuous in 46% of patients, recurrent in 27%, continuous with recrudescence in 27%. 81% of patients presented skin involvement during their disease course, 58% neurological, 53% musculoskeletal, 41% gastrointestinal, 38% of lymphoid organs, 24% haematological, 17% cardiovascular, 14% ocular and 5% genitourinary. In 4 patients a neoplasm occurred.
Synthetic DMARDS were used in 34 patients. Azathioprine and cyclophosphamide, used in 10 and 3 patients respectively, were withdrawn for inefficacy; thalidomide, used in 3 patients, was withdrawn for side effects. Mofetil mycophenolate was used in 6 patients, still ongoing at last follow-up in 2, while methotrexate in 14 patients, still ongoing at last follow-up in 8, of those 5 associated to anti-TNF.
76 patients (82%) received treatment with anti-TNF: 66 patients were treated with etanercept (in 63 treatment was ongoing al last follow-up), 17 patients with adalimumab (in 10 treatment was ongoing at last follow-up). IL-1 and 6 inhibitors were used in 7 and 1 patients respectively, withdrawn for inefficacy.

24 adverse events were reported, while on treatment; of these, 11 were serious: 2 were disease-related, 3 resolved with surgery, 4 were infections and one (hepatic nodular hyperplasia) required drug-change. One patient attempted suicide while on biological treatment.
79% of patients achieved a complete control of the disease during follow-up.. 2 patients died: one for sepsis at the age of 52, one for lung cancer at the age of 28.

Conclusion: The study analyses a large series of DADA2 patients with prolonged follow-up, confirming the clinical heterogeneity of this condition and the difficulty in the molecular diagnosis. Anti-TNF drugs confirms their efficacy and good safety profile in this condition.

Disclosure of Interest: None declared

Identifier: PT79

PRECLINICAL EVALUATION OF LENTIVIRAL GENE THERAPY FOR THE TREATMENT OF DADA2: ENGRAFTMENT AND BIODISTRIBUTION STUDIES IN HUMANISED NBSGW MICE

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Introduction: Deficiency of adenosine deaminase type 2 deficiency (DADA2) is caused by bi-allelic loss-of-function mutations in *ADA2*. While anti-TNF therapy is effective for the autoinflammatory and vasculitic components of the disease it does not correct marrow failure or immunodeficiency. Allogeneic stem cell transplantation (HSCT) offers a potential cure is limited by challenges such as graft versus host disease and donor availability. Our previous preclinical studies demonstrated that lentiviral-mediated ADA2 gene therapy, could restore ADA2 enzyme activity in patient-derived cells, correct macrophage inflammatory activation and reduce endothelial activation *ex vivo.*
Objectives: The objective of this study was to evaluate the biodistribution and engraftment potential of lentiviral-mediated ADA2 gene therapy in healthy donor- and DADA2 patient-derived hematopoietic stem cells (HSC) *in vivo*.
Methods: A humanized NBSGW mouse model (NOD, B2mnull, SCID, IL2Rγnull) was used to evaluate the engraftment potential and biodistribution of lentivirally transduced HSC. Healthy donor-derived and DADA2 patient-derived hematopoietic stem cells (HSC) were transduced with a lentiviral vector containing the ADA2 gene. Transduction efficiency and engraftment were evaluated by PCR analysis to detect viral integration, along with histological assessments of non-hematopoietic organs to identify potential adverse tissue changes. The multilineage differentiation and engraftment capacity of the transduced HSC were monitored in vivo, and functional assessment of ADA2 enzyme activity was conducted to confirm therapeutic restoration in the patient-derived HSC.
Results: Lentiviral transduction of healthy donor HSC successfully preserved their multilineage differentiation and engraftment capacity in the NBSGW mice, with no adverse effects on HSC functionality post-transplantation. PCR analysis confirmed the absence of viral integration in non-hematopoietic organs, ensuring the precision and safety of the approach. Histological evaluations showed no abnormal tissue changes in any of the organs. In DADA2 patient-derived HSC, ADA2 transduction resulted in restored enzyme expression, suggesting an improvement in cellular function. Additionally, transduced patient-derived HSC showed enhanced engraftment potential, further supporting the therapeutic promise of this approach for DADA2.
Conclusion: These findings lay a strong foundation for further clinical development of ADA2 gene therapy as a potential curative treatment for DADA2, advancing toward clinical application.
Acknowledgments: Action Medical Research, Lifearc grant, Rosetrees Trust and GOSH Children's Charity.
Disclosure of Interest: None declared

Identifier: PO012

ASSESSMENT OF C26:0 LYSOPHOSPHATIDYLCHOLINE AND CHITOTRIOSIDASE LEVELS IN PATIENTS WITH DEFICIENCY OF ADENOSINE DEAMINASE 2

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Introduction: Deficiency of adenosine deaminase 2 (DADA2) is a monogenic autoinflammatory disease caused by biallelic genetic variants associated with loss of function in the ADA2 gene. One of the recently proposed hypotheses for the role of ADA2 protein suggests that it is a lysosomal enzyme, with DNAse activity (1). In Aicardi-Goutières Syndrome (AGS), a prototype inborn error of the nucleic acid metabolism, an increase in the levels of C26:0 lysophosphatidylcholine (C26:0 LPC) has been reported (2). Furthermore, in several lysosomal diseases, the levels of chitotriosidase (ChT), an enzyme produced by activated macrophages, are significantly increased (3). Data on the expression of C26:0 LPC and chitotriosidase in DADA2 are still lacking.
Objectives: To evaluate the levels of C26:0 LPC and chitotriosidase in individuals with DADA2.
Methods: We collected dried blood spot (DBS) samples from patients with DADA2. C26:0-LPC, levels were determined in the DBS samples by high-performance liquid chromatography coupled to tandem mass spectrometry, along with the levels of adenosine (Ado) and deoxyadenosine (dAdo). ChT levels were determined in the DBS using a using fluorescent substrate, as previously described (4). All analyses were performed in duplicate. The Ethics Committee of the Hospital de Clinicas de Porto Alegre approved the project (#DIPE 2024-0101).
Results: Three patients with DADA2 were included in this study, all with an inflammatory and vasculitic phenotype (all females, with ages of 34, 25, and 23 years). All subjects were on anti TNF treatment (etanercept), although with incomplete symptomatic resolution. The levels of all evaluated biomarkers were in the normal range (table 1).

Table 1 – Levels of DBS biomarkers in three patients with DADA2

	Subject 1	Subject 2	Subject 3	Reference range
Ado	0.24	0.28	0.21	<3.42 µmol/L
dAdo	0.01	0.01	0.01	<0.03 µmol/L
C26:0 LPC	0.22	0.25	0.32	<0.78 µmol/L
ChT	21.1	24.4	20.1	7.0-89.9 nmol/h/mL

Conclusion: The normal ranges of Ado and dAdo in this sample are in accordance with previous reports and are compatible with a biological role for ADA2 not directly related to its deaminase activity. The results of this study also

suggest that C26:0-LPC and ChT are not highly sensitive biomarkers for vasculitic DADA2. As a next step, we intend to evaluate other biomarkers related to the presumed lysosomal DNAse activity of ADA2.

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Disclosure of Interest: None declared

Identifier: PO086

DEFICIENCY OF ADENOSINE DEAMINASE 2: A TALE OF TWO PATIENTS, ONE MUTATION

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Introduction: Deficiency of adenosine deaminase 2 (DADA2) is an autosomal recessive disorder caused by mutations in the ADA2 gene. First described in 2014, it manifests as a monogenic vasculopathy or vasculitis that primarily affects children. The condition resembles polyarteritis nodosa (PAN) and exhibits considerable clinical variability, ranging from mild symptoms to severe vasculitis and hematologic abnormalities.

Objectives: This case presentation aims to discuss two patients with the same genetic mutation in the ADA2 gene, but differing clinical manifestations of DADA2.

Methods: We analyzed the clinical and laboratory findings of both patients to highlight the variability in presentation of DADA2.

Results: *Case 1*:

A 13-year-old Azerbaijani male presented with fever, malaise, abdominal pain, weight loss, and diarrhea. Physical examination revealed pale skin, high blood pressure, and livedo racemosa on his abdomen and legs. Neurologic examination was notable for left eye esotropia (present since age 3). Abdominal tenderness was present, but no organomegaly or lymphadenopathy was observed. Laboratory tests showed neutrophilic leukocytosis, thrombocytosis, elevated C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and mildly elevated ferritin. Abdominal CT angiography revealed multiple aneurysms in the renal, splenic, ileocolic, and jejunal arteries. Cranial MR angiography identified aneurysms in the external carotid and temporal arteries. These findings led to a prediagnosis of PAN.

The patient was started on pulse steroid therapy and antihypertensive medications, but no improvement was seen after five doses of steroids and one dose of cyclophosphamide. A seizure occurred on the sixth day, though MRI showed no intracranial hemorrhage. Splenic vein embolization was required due to hemorrhage from a splenic artery aneurysm. Suspecting DADA2, we tested ADA2 enzyme activity, which showed no enzyme function. Genetic testing revealed a pathogenic homozygous c.752C>T p.(Pro251Leu) mutation in the ADA2 gene, confirming DADA2. Treatment with anti-TNF therapy was initiated, and family members were advised to undergo genetic screening.

Case 2:

A 6-year-old female presented with a two-month history of prolonged fever and arthralgia, specifically in the wrists. There were no associated symptoms such as weight loss, night sweats, or cough. Physical examination showed a mild livedoid rash on the legs, and mild cervical lymphadenopathy, but no signs of arthritis or organomegaly. Laboratory tests revealed neutrophilic leukocytosis, mild microcytic anemia, thrombocytosis, and elevated CRP and ESR. Viral serologies, ANA, anti-dsDNA, and tuberculin skin tests were all negative, and C3, C4, and immunoglobulin levels were normal. Abdominal, renal, and portal vein Doppler ultrasonography were unremarkable. Bone marrow aspiration showed no signs of malignancy.

Despite starting colchicine therapy, there was no clinical improvement. Genetic testing revealed the same pathogenic c.752C>T p.(Pro251Leu) mutation in the ADA2 gene. DADA2 was diagnosed, and anti-TNF therapy was initiated. MR angiographies were planned to assess potential vasculitis. The patient's family was also referred for genetic counseling.

Conclusion: DADA2 presents a broad clinical spectrum, with significant variability between patients. It should be suspected in any child with a stroke, livedoid rash, unexplained cytopenias, or rheumatic symptoms. The heterogeneity of DADA2 cannot be fully explained by low ADA2 activity and genetic factors alone, suggesting the importance of

epigenetic factors in disease presentation. It is crucial to emphasise that DADA2 can manifest in a wide spectrum of clinical forms, and the same genotype can manifest in two very different phenotypes.

Disclosure of Interest: None declared

Identifier: PO107

COMBINATION OF BIOLOGICS AND JAK INHIBITORS IN THE TREATMENT OF REFRACTORY SYSTEMIC AUTOINFLAMMATORY DISEASES

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Introduction: Systemic autoinflammatory disorders (SAIDs) result from genetic defects in innate immunity, leading to excessive activation of inflammatory pathways such as IL-1, TNF, and JAK/STAT. Symptoms range from fever to life-threatening conditions like encephalitis and AA amyloidosis. Treatment focuses on controlling inflammation with glucocorticoids, colchicine, DMARDS, and advanced drug monotherapies (biologics and JAK inhibitors). Advanced combination treatment (ACT) refers to combining two or more biologics or a biologic and a JAK inhibitor to treat inflammatory diseases.

Objectives: To present our experience using ACT in difficult-to-treat SAID patients.

Methods: The charts of genetically confirmed SAIDs or undifferentiated autoinflammatory diseases (uSAID) who received two or more advanced treatments simultaneously were retrospectively reviewed. Collected data included patient demographics, clinical outcomes, prior therapies, and adverse events. The treatment responses were evaluated using a composite score that included steroid-sparing effects, improvements in C-reactive protein (CRP) levels, and the Clinical Global Impression-Improvement (CGI-I) scale. Treatment outcomes were categorized as non-response, partial response, or complete response, with the study evaluating the safety and efficacy of ACT in this patient cohort.

Results: The study included 38 patients with SAIDs who received ACT, with a median age of 30 years (range: 4–76). The most common conditions requiring ACT were pyoderma gangrenosum, pyogenic arthritis and acne syndrome, mevalonate kinase deficiency, and uSAIDs. Nearly all patients had complications such as joint destruction, skin disfigurement, retinopathy, and dependence on glucocorticoids or opioids. A total of 65 ACT regimens were trialed, with IL-1 and TNF inhibitor combinations being the most frequently used. ACT resulted in 22 complete responses, 29 partial responses, and 14 non-responses. Treatment was discontinued in 38 regimens due to inefficacy, secondary loss of efficacy, or adverse events. At the final assessment, 68% of patients remained on ACT, with a median treatment duration of 60 months (range: 11–186). Overall, ACT yielded significant clinical and laboratory improvements for most patients. Sixteen serious adverse events (all infections) were reported during 151.9 patient-years of follow-up, with no deaths attributed to ACT.

Conclusion: ACT demonstrates substantial clinical benefits for patients with refractory SAIDs, though challenges such as secondary loss of efficacy and infection risk persist.

Disclosure of Interest: None declared

Identifier: PO114

TYPE I INTERFERON SCORE AS A BIOMARKER OF DISEASE ACTIVITY IN ADA2 DEFICIENCY

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Introduction: Deficiency of adenosine deaminase 2 (DADA2) is a rare systemic autoinflammatory disease with autosomal recessive inheritance caused by biallelic loss of function mutations in the ADA2 gene. The phenotypic spectrum of the disease is broad, including fever, early-onset vasculitis, stroke, immunologic and hematologic dysfunction. If not recognized and treated the disease can cause mortality and serious long-term sequelae. Although the exact mechanism involved in the pathogenesis of the disease is still unclear, in patients with DADA2 an increased type I IFN score (IS), correlated with disease activity and response to treatment, has been demonstrated. Recently an activity and damage score (DADA2AI and DADA2DI) have been developed[1] to aid in the longitudinal assessment of these patients, inter- and intra-patients comparisons and in the evaluation of long term outcome with different therapies

Objectives: To apply the recently published DADA2AI and DADA2DI in a bicentric cohort of DADA2 patients and to evaluate whether longitudinal follow-up of type I IS reflects disease activity and could potentially be used as a biomarker of the disease

Methods: ADA2 enzymatic activity was measured in a colorimetric assay adapted from Giusti et al (1974). An HEK293T overexpression system was setup to evaluate the impact of suspected pathogenic ADA2 variants on protein expression/secretion and enzymatic activity. Real time PCR assays were performed on whole blood cells, the expression levels of 6 interferon-induced genes was evaluated and the IS was calculated. DADA2AI was calculated based on criteria described by Bucciol et al.

Results: 14 patients from 11 families were included. All patients carried 2 pathogenic ADA2 variants and showed reduced serum ADA2 activity. Twelve patients presented with an inflammatory vasculitis phenotype while 2 had an haematological phenotype. One patient showed hypogammaglobilinemia. The enzymatic activity of ADA2 variants in an HEK2937 overexpression system correlated with residual enzymatic activity measured in patient serum samples. Longitudinal measurement of type I IS was performed in all patients in combination with assessment of DADA2 activity index. In our cohort, IS score was only slightly elevated in the majority of patients (median value of 4.055 versus normal IS<2.05) and did not correlate with DADA2AI or with routine laboratory parameters. In particular the IS did not normalize during TNF-I treatment. The DADA2DI remained stable or decreased in all patients with the inflammatory vasculitis phenotype treated with TNF-inhibitor, thus confirming the efficacy of this treatment in preventing new damage/sequelae

Conclusion: The DADA2AI and DADA2DI can be easily calculated from the patients charts and are valid and reproducible instruments to monitor patient's disease activity and response to treatment. The pathogenic mechanism in DADA2 is not entirely understood: type I IFN pathway activation is probably part of a much more complex mechanism and does not appear to be TNF-dependent. In our cohort, the 6gene type I IFN score did not prove to be a good biomarker for longitudinal follow-up of DADA2 disease activity and response to treatment

1 Bucciol et al, J. Clin Immunol. 2023; 2 Rice et al J. Clin Immunol. 2017

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Identifier: PO129

NEUTRALIZING ANTIBODIES AND ANTI-TUMOR NECROSIS FACTOR (TNF) MONOCLONAL ANTIBODY MEDICATIONS

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Introduction: Use of anti-TNF biologics has been a major advance in the treatment of patients with genetic autoinflammatory conditions. Anti-TNF monoclonal antibodies such as *infliximab, golimumab, certolizumab and adalimumab as well as the soluble receptor fusion protein, etanercept, have been used in genetic autoinflammatory diseases with significant positive impact. As much as we have come to know about these medicines,* less is known about the propensity of these agents to stimulate the production of antibodies against themselves and the clinical implications it has on patients if not used with disease modifying anti-rheumatic drugs (DMARDS) such as methotrexate (MTX) or azathioprine (AZA) to block the development of anti-drug antibodies.
Objectives: To investigate the risk of neutralizing antibody production against TNF-alpha blocking medications in patients with autoinflammatory diseases if not used in conjunction with DMARDs.
Methods: A case report of 6 autoinflammatory patients; 3 with deficiency of adenosine deaminase 2 (DADA2), 1 with familial Mediterranean fever (FMF) and 2 with undifferentiated autoinflammatory disease (UAD), none of whom were on a DMARD at the time of TNF-inhibitor initiation. All patients began to manifest symptoms of their disease on their current treatment with a TNF-inhibitor. All patients were evaluated and parameters of inflammatory disease activity were measured (C-reactive protein, erythrocyte sedimentation rate, ferritin and complete blood count). Drug-induced anti-TNF-alpha blocker antibodies were analyzed using ELISA.
Results: All 6 patients started to develop clinical symptoms which prompted the clinical team to test for anti-drug antibodies. They were all positive for anti-TNF antibodies. diagnosis of DADA2 was on adalimumab and developed a bilateral leg rash. They were switched to golimumab and MTX was added with good outcome and resolution of leg rash. Patient 2 with a diagnosis of DADA2 was on adalimumab and developed loss of consciousness and E. nodosum rash. They were switched to golimumab and MTX with resolution of symptoms. Patient 3 with a diagnosis of DADA2 was on adalimumab and developed skin lesions. They were switched to golimumab and MTX with resolution of symptoms. Patient 4 with a diagnosis of FMF was on infliximab and developed severe abdominal pain and blood in their stool. They were switched to adalimumab with MTX and symptoms resolved. Patient 5 with a diagnosis of FUO was on adalimumab and developed ulcerative colitis and PG lesions. Patient 6 with a diagnosis of FUO was on infliximab and AZA with ongoing disease activity. Recommendation was made to change to golimumab with AZA but they decided to remain on infliximab because they were getting some relief with the current dose of infliximab.
Conclusion: The presence of drug-induced neutralizing antibodies to TNF-α blockers has been associated with worse clinical response as evidenced by all 6 patients experiencing disease associated symptoms whilst neutralizing antibodies were present. The data support the use of DMARDs such as MTX or AZA to help prevent neutralizing antibodies in patients with autoinflammatory diseases.
Disclosure of Interest: None declared

Identifier: PO134

DADA2: THE FIRST REPORT OF A MULTICENTER EGYPTIAN EXPERIENCE

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Introduction: Adenosine Deaminase 2 deficiency (DADA2) is a rare autoinflammatory disorder first described in 2014. DADA2 arises from mutations in the ADA2 gene, leading to diminished ADA2 enzyme activity. Although DADA2 is considered rare, the global distribution of DADA2 is variable, and higher incidence rates in certain ethnic groups are expected. Collaborative efforts are crucial for elucidating the complex epidemiology of this disorder. Over the past few years, we have diagnosed several DADA2 patients in different University hospitals across Egypt.

Objectives: We aim to report the clinical and laboratory characteristics of a cohort of Egyptian children with DADA2

Methods: This cross-sectional study included patients from families with DADA2 diagnosed in several Pediatric University hospitals in Egypt. The data was collected from patients' files after approval of the ethics committee of the faculty of medicine, University of Alexandria; the patients' confidentiality was preserved. Any patient with symptoms suggestive of DADA2 was screened using ADA2 enzyme assay or molecular diagnosis. The patients were screened if they fell into one of these categories: first, patients with immunedysregulation/autoinflammatory manifestations. Second, patients with pure red cell aplasia (PRCA) or bone marrow failure (BMF) of unclear etiology, and the third category included patients with unexplained stroke or recurrent thrombosis, especially if associated with immunological or hematological manifestations. Clinical and laboratory data of the patients were analyzed.

Results: In total, 113 families were screened. In seven families, a molecular diagnosis was made first, either through an autoinflammatory panel or whole exome sequencing for immunedysregulation symptoms, and in one family for PRCA and stroke. The rest of the families were first screened using an ADA2 enzyme assay if they fell into one category of symptoms suggestive of DADA2. Some of these patients have been mistakenly diagnosed with familial Mediterranean fever or other periodic fever syndromes, thrombotic thrombocytopenic purpura, very early-onset inflammatory bowel disease, and Diamond-Blackfan syndrome. We recruited patients from six governorates from Upper Egypt and the Delta region. In total, we have diagnosed 15 families with 27 DADA2 patients and 40 carriers. The range of ADA2 enzyme levels in the affected individuals was from 0 to 0.96 IU/ml, while in the carriers, it ranged from 2.32 to 14.72 IU/ml with a median of 4.96 IU/ml; in four individuals, the ADA2 enzyme assay was in the normal range despite a confirmed heterozygous state. Four previously unreported ADA2 variants were identified. All but one homozygous patient (sister of a symptomatic child) had symptoms of DADA2, and some of the carrier individuals also have symptoms that may be attributed to DADA2. After confirmation of the diagnosis, several patients were started on anti-TNF therapy with a good response. Two sisters who presented with bone marrow failure underwent a successful hematopoietic stem cell transplant from their fully matched mother.

Conclusion: Although DADA2 is a rare disease, it is important to report the epidemiology of the disease in different regions of the world. Our population is underrepresented, which explains why we found several new ADA2 variants. The opportunity for international collaboration for ADA2 enzyme assay and molecular testing helped to confirm the diagnosis in these patients. Implementing targeted therapy improved the patients' condition. Spreading awareness among pediatricians is essential to increase the rate of diagnosis of DADA2 and prevent complications.

Disclosure of Interest: None declared

Identifier: PO135

PERIODONTITIS IN DADA2 PATIENTS WITH SEVERE NEUTROPENIA

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Introduction: Deficiency of adenosine deaminase 2 (DADA2) is an autosomal recessive disease caused by pathogenic mutations in *ADA2.* Severe and refractory neutropenia is a hematologic feature in the phenotypic spectrum of DADA2. Periodontal disease is often recognized as a manifestation of severe neutropenia, but not previously highlighted in neutropenic DADA2 patients.
Objectives: To describe five cases of periodontal disease in DADA2 patients affected by severe neutropenia.
Methods: Case report of five DADA2 patients identified with known periodontal disease and severe neutropenia in the NIH DADA2 cohort. Medical records from 12/18/2005 to 12/01/2024 were reviewed.
Results: Patient 1 (R169Q/deletion in exon 7) is a 57-year-old edentulous female with a 28-year history of neutropenia (ANC = 0), fevers, cervical lymphadenopathy, and splenomegaly with splenectomy. No known history of strokes. Periodontal disease manifested with chronic tooth decay, gingival inflammation, severe mandibular regression, dental extractions, and poor osseointegration of dental implants. Dental imaging demonstrated severe bone loss. The patient was treated with cyclosporine, glucocorticoids, and a TNF-inhibitor induction dose of infliximab, followed by adalimumab, with positive treatment response and neutrophil count normalization (ANC = 2.07). No recurrence of periodontitis after blood count stabilization has been reported.
Patient 2 (H112Q/R34W) is a 44-year-old female with a history of transient neutropenia (ANC = 0.8), Raynaud's, fevers, arthralgia, genital ulcers, livedo reticularis, splenomegaly, and recurrent non-healing skin ulcers in both lower extremities. No history of strokes. Periodontal disease manifested by extensive painful oral ulcerations and mucositis which correlated with episodes of severe neutropenia. She was treated with adalimumab for DADA2 with resolution of neutropenia and no recurrence of oral ulcers and fevers.
Patient 3 (R169Q homozygous) is a 43-year-old female with a history of neutropenia (ANC = 0), Raynaud's, two episodes of central retinal artery occlusions (resulting in right eye blindness), fevers, headaches, arthralgia, livedo reticularis, lymphadenopathy, esophageal necrosis, and common variable immunodeficiency. Periodontal disease was manifested by oral ulcers and painful gingivitis. She was treated with etanercept for DADA2, response was not observed due to medication discontinuation after patient's decision to withdraw all medical care. Patient expired 07/2024.
Patient 4 (H112Q/R169Q) is a 26-year-old male with a history of neutropenia (ANC = 0.3), fevers, skin rashes, and splenomegaly. Periodontal disease was manifested by periodontal abscess and oral ulcers. No history of strokes. Patient received a hematopoietic stem cell transplant with no known recurrence of neutropenia or periodontal issues.
Patient 5 (R169Q/G47W) is a 23-year-old male with a history of neutropenia (ANC = 0), lymphadenopathy, arthralgia, myalgia, polyarteritis nodosa, fevers, and hepatomegaly. Periodontal disease was manifested by oral ulcers. No history of strokes. Patient was initially treated with filgrastim with short term improvement of neutropenia and oral ulcers. Patient received a hematopoietic stem cell transplant with eradication of DADA2 and no oral ulcer recurrence.
Conclusion: These case reports highlight periodontitis in the severe neutropenia of DADA2. We hypothesize that DADA2 neutropenia induces IL-23 and IL-17 release by resident macrophages in the gingiva, similar to leukocyte adhesion deficiency (which may be treatable with Ustekinumab). Since the neutropenia of DADA2 is at least partially due to destruction of precursors in the bone marrow, local cytokine production is unabated, leading to hyperinflammation. To further understand the mechanisms leading to periodontitis in neutropenic DADA2, we plan to conduct careful assessments of DADA2 patients' oral history/dental exams, and when appropriate, gum biopsies.
Disclosure of Interest: None declared

Identifier: PO169

PERIPHERAL ANEURYSMS IN A PATIENT WITH DEFICIENCY OF ADENOSINE DEAMINASE 2 (DADA2)

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Introduction: Deficiency of Adenosine Deaminase 2 (DADA2) is the first identified monogenic vasculitis syndrome at the molecular level. It results from biallelic hypomorphic mutations in the ADA2 gene, which encodes the adenosine deaminase 2 (ADA2) enzyme. The condition is characterized by a spectrum of vascular abnormalities, ranging from livedo reticularis to polyarteritis nodosa (PAN), as well as severe ischemic or hemorrhagic strokes that can be life-threatening. The associated vasculitis and inflammatory processes affect multiple organ systems, accounting for gastrointestinal, hepatic, and renal manifestations (1).

Objectives: To the best of our knowledge peripheral aneurisyms has not been reported in DADA2 before. Herein, we present a patient with giant peripheral arterial aneurisyms.

Methods: a 24-year-old woman presented in 2007 with two episodes of massive lower gastrointestinal bleeding of unknown origin at the age of thirteen. Following stabilization, the patient was referred to gastroenterology, rheumatology, and cardiovascular surgery departments for further evaluation. She exhibited positive Raynaud's phenomenon, elevated ESR and CRP levels, and polyarthritis of hands. Digital ischemic ulcers were noted on the hands and feet. Initial investigations, including ANA and ENA profile, were negative. The patient was started on 4 mg/day methylprednisolone, hydroxychloroquine and nifedipine.

Between 2007 and 2017, the patient exhibited recurrent ischemic and ulcerative skin lesions, requiring treatment with azathioprine, hydroxychloroquine, leflunomide, and methylprednisolone without a significant benefit. Due to ischaemic symptoms an angiographic examination was performed in 2017 revaling multiple ~1 cm aneurysms at intrarenal and intrasplenic regions. A pseudoaneurysm approximately 4 cm in size, caused by aneurysm rupture and extravasation, was detected in the right axillary region, along with millimeter-sized aneurysms in small vessels in solid organs. Further evaluations ruled out ANCA-associated vasculitis and Behçet's disease.

Results: Genetic analysis was performed with a presumtive diagnosis of ADA2 deficiency yielded c.139G>C homozygos variant. Persistent active vasculitis and non-responsive digital ischemia led to the initiation of etanercept. The patient remained stable until 2021 but subsequently experienced persistent nausea, headache, and dizziness. Brain MRI findings showed microhemorrhages and features consistent with chronic small vessel occlusion and cerebrovascular accident, prompting a switch to certolizumab.

Conclusion: ADA2 deficiency is a rare systemic vasculopathy that manifests with recurrent ischemic complications, however, to the best of our knowledge and aneurisym formation has not been reported before. Early genetic testing and early treatment with anti-TNF agents are crucial for stabilizing disease progression.

Acknowledgments: References

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Figure 1 : A) An aneurysm or pseudoaneurysm approximately 8x6.5x7 cm in size, extending posteriorly to the pectoral muscle planes in the right axillary region, is thought to originate from the descending scapular artery. **B)** Same plane at BT image after treatment

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DATA FROM THE EUROFEVER REGISTRY FOR CENTRAL AND EASTERN EUROPEAN COUNTRIES: AN UPDATE

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Introduction: Autoinflammatory diseases (AID) are rare chronic conditions characterized by abnormal regulation of innate immunity. In countries with small populations, only few patients with AID are reported, making it impossible to carry out research and deepen the understanding of AID. To overcome this shortcoming, in 2010, a worldwide registry – the Eurofever registry was founded. The main aim was to link together all European centres that follow patients with AID. The first, and so far, the last demographic data from Central and Eastern European countries from the first 18 months of enrolment in the Eurofever registry was published in 2012.

Objectives: To collect and analyse AID demographic data for Central and Eastern European countries currently available in the Eurofever registry.

Methods: We requested data from the Eurofever registry for 16 Central and Eastern European countries – Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Macedonia, Montenegro, Poland, Romania, Serbia, Slovakia, and Slovenia. We included data on gender, diagnosis, comorbidities, genetics, and age at the start of the symptoms, at diagnosis, and initial visit to the tertiary centre. The collection of the data and statistical analysis was done using the IBM SPSS Statistics (version 29.9.2.0).

Results: On October 29th, 2024, we received data for 11 of the 16 requested centres; Macedonia and Montenegro had no registered centres, and no data were available for Albania, Bosnia and Herzegovina, and Estonia. Within the other centres, 554 patients were identified: 5 (0.9 %) in Bulgaria, 36 (6.5%) in Croatia, 176 (31.8%) in the Czech Republic, 2 (0.4%) in Hungary, 4 (0.7%) in Latvia, 2 (0.4%) in Lithuania, 7 (1.3%) in Poland, 47 (8.5%) in Romania, 5 (0.9%) in Serbia, 247 (44.6%) in Slovakia, and 23 (4.2%) in Slovenia. Of these, 302 (55%) were male, and 528 (95%) were Caucasian. Thirty-six (6.5%) had co-morbidities, ranging across different systems of the body. We recorded 13 different diagnoses; the exact data is presented in Table 1. For 193 (35%), genetic analysis was available, results were positive for 159 (82%), negative for 9 (5%), and non-informative for 25 (13%) patients.

Table 1: Autoinflammatory disease diagnoses among patients

Diagnosis	N (%)	Diagnosis	N (%)
Behcet's disease	11 (2.0)	NLRP12-related disease	1 (0.2)
Blau's disease	1 (0.2)	PAPA[f]	13 (2.3)
CAPS[a]	25 (4.5)	PFAPA[g]	267 (48.2)
CRMO[b]	37 (6.7)	SURF[h]	36 (6.5)
DADA2[c]	7 (1.3)	TRAPS[i]	18 (3.2)
FMF[d]	117 (21.1)	Undefined	1 (0.2)
MKD[e]	20 (3.6)		

a CAPS – cryopyrin-related periodic syndromes, [b] CRMO – chronic recurrent multifocal osteomyelitis, [c] DADA2 – deficiency of adenosine deaminase 2, [d] FMF – familial mediterranean fever, [e] MKD – mevalonate-kinase deficiency, [f] PAPA – pyogenic sterile arthritis, pyoderma gangrenosum and acne syndrome, [g] PFAPA – periodic fever, aphthous stomatitis, pharyngitis, adenitis, [h] SURF – syndrome of undifferentiated recurrent fever, [I] TRAPS – tumor necrosis factor receptor-associated periodic syndrome

Identified patients ranged in age from zero to 60 years, with a median age of 2.5 years (IQR 1.0-6.4 years) at the start of symptoms, 5.6 years (IQR 2.9-14.0 years) at the time of diagnosis and 5.9 years (IQR 3.1-14.0 years) at the time of the initial visit to the tertiary centre. The diagnosis of AID was determined before coming to the tertiary centre in 118 (21%) patients. The median time from the start of the symptoms to the initial visit to the tertiary centre was 2.9 years (IQR 0.9-8.7 years). If the diagnosis had not been established before the initial visit, it was usually made during this visit, with a median time from the initial visit to the diagnosis of 0 days (IQR 0-134 days).

Conclusion: We report the most recent demographic data for 554 patients with AID from 11 Central and Eastern European countries from the Eurofever registry.
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