Treatment and outcome in deficiency of adenosine deaminase 2: a literature review

Running title: DADA2 treatment and outcome

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Abstract

Objectives: Deficiency of adenosine deaminase 2 (DADA2) is a rare disease with varying phenotypes and disease outcomes. We aimed to summarize the treatments of DADA2 and to explore the factors associated with disease outcome.

Methods: A systemic literature review of DADA2 was conducted. Cases were included if they had documented detailed genotypes, phenotypes, treatment protocols and outcomes. Patients were categorized into uncontrolled and controlled groups. Factors associated with disease outcome were analyzed with logistic regression models.

Results: A total of 242 DADA2 patients with treatment protocols and responses were included, 17 of whom required no treatment. The general effective rate of TNFi was 78.6% (103/131). Hematological abnormalities and increased acute phase reactants are independently associated with TNFi effectiveness, OR=0.21 (95%CI 0.07-0.661, p=0.007) and 9.62 (95%CI 2.31-40.00, p=0.002), respectively. Among those 225 patients requiring active treatment, 157 (69.8%) patients were in the controlled group, and 68(30.2%) in the uncontrolled group. Neither age of disease onset nor genotype was associated with disease outcome. Increased acute phase reactants (APRs), constitutional symptoms, neurological symptoms, and treatment with TNFα inhibitors (TNFi) were independently associated with disease control, while recurrent infections and severe vascular events were the main causes of mortality (10/21 and 6/21, respectively).

Conclusion: In patients requiring treatment, symptoms of systemic inflammation and vasculitis and TNFi treatment are associated with disease control; while recurrent infections and severe vascular events should be treated intensively as they are the main causes of death. Hematological abnormalities should be monitored as it would decrease TNFi effectiveness.

Key words: Deficiency of adenosine deaminase 2, Treatment, Outcome, TNFα inhibitor.

Resumen

Objetivos: El déficit de adenosina desaminasa 2 (DADA2) es una enfermedad rara con diferentes fenotipos y una evolución variable de la enfermedad. Nuestro objetivo es resumir los tratamientos de DADA2 y explorar los factores asociados con la evolución de la enfermedad.

Métodos: Se realizó una revisión bibliográfica sistémica de DADA2. Los casos se incluyeron fueron aquellos que habían documentado el genotipo, fenotipos, protocolo de tratamiento y evolución. Los pacientes se clasificaron en grupos controlados y no controlados. Los factores asociados con la evolución de la enfermedad se analizaron con modelos de regresión logística.

Resultados: Se incluyeron un total de 242 pacientes DADA2 con los protocolos de su tratamiento y la respuesta al mismo; 17 de los cuales no requirieron tratamiento. La eficacia general de los inhibidores de TNF-α (TNFi) fue del 78,6% (103/131). Las anomalías hematológicas y el aumento de los reactantes de fase aguda se asociaron de forma independiente con la eficacia del TNFi, OR = 0,21 (IC del 95%: 0,07 a 0,661, p = 0,007) y 9,62 (IC del 95%: 2,31 a 40,00, p = 0,002), respectivamente. Entre los 225 pacientes que requirieron tratamiento activo, 157 (69,8%) pacientes estaban en el grupo controlado y 68 (30,2%) en el grupo no controlado. Ni la edad de inicio de la enfermedad ni el genotipo se asociaron con la evolución de la enfermedad. El aumento de los reactantes de fase aguda (APR), el deterioro constitucional, los síntomas neurológicos y el tratamiento con TNFi, se asociaron de forma independiente con el control de la enfermedad, mientras que las infecciones recurrentes y los eventos vasculares graves fueron las principales causas de mortalidad (10/21 y 6/21, respectivamente).

Conclusión: Los síntomas de inflamación sistémica, la vasculitis y el tratamiento con TNFi se asociaron con el control de la enfermedad en aquellos pacientes con DADA2 que requirieron tratamiento. Las infecciones recurrentes y los episodios vasculares graves deben tratarse de forma rápida y adecuada, ya que fueron las principales causas de muerte. Asimismo, se deben controlar las alteraciones hematológicas, ya que disminuyen la eficacia de los TNFi.

Palabras clave: Déficit de adenosina desaminasa 2, Tratamiento, Evolución, inhibidores de TNF-α.

Introduction

Deficiency of adenosine deaminase 2 (DADA2) is an inherited autoinflammatory disease caused by mutations in the *ADA2* gene (adenosine deaminase 2, OMIM: *607575), formerly known as *CECR1 (cat eye syndrome chromosome region candidate 1) [1, 2]*. ADA2, predominantly expressed by monocytes, functions to regulate the innate immune response, maintain vessel integrity, and participate in endothelial cell and hematopoietic cell proliferation [3]. ADA2 loss of function would lead to chronic upregulation of neutrophil activity, dysregulate neutrophil extracellular trap formation (NETosis) and reduce M2 macrophage differentiation [4-6]. These pathophysiological changes reduce endothelial cell integrity and activate a bundle of inflammatory responses, including CD14+ inflammatory monocytes, interferon (IFN) and nuclear factor-κB (NF-κB) signaling and intracellular production of IL-1β, IL-6 and TNF cytokines [2, 7, 8]. Particularly, TNFα is the most important cytokine as it penetrates the involved vessels and released into peripheral serum, inhibition of which could decrease both IFN and NF-κB inflammatory signatures, reduce perivascular TNF, resolute inflammatory myeloid cell infiltrates and normalize blood vessels and endothelial layers [2, 7, 8]. Furthermore, abrogated B cell proliferation and reduced secretion of IgM and IgA were also noted in patients with DADA2 [1].

Genetically, the culprit mutations have been reported over the entire coding region of ADA2, including missense mutations, nonsense mutations, deletions, and splicing variants (reviewed by Meyts et al [9]). Clinically, DADA2 contains three symptom domains: vasculopathy and inflammation, hematological abnormalities and immunodeficiency [3, 10]. The disease course varies greatly from asymptomatic to rapidly progressive. Some patients died before the diagnosis [1, 2, 11], and other patients survived with major disability and irreversible organ damage [12, 13]. Some have suggested profound gene structure damage and undetectable ADA2 activity tent to be associated with disease severity [14], while studies on disease outcome were lacking. Tumor necrosis factor α inhibitors (TNFis) have significantly improved disease outcome, but there are still patients who do not respond to TNFis or even die while taking them [15-17]. Our previous review showed that patients with DADA2 of different age groups (pediatric and adult onset) and genotypes had contrasting phenotypes but similar responses to steroids and TNFis [18]. Thus, we aimed to evaluate the treatment options reported in DADA2

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and identify the factors affecting treatment response.

Materials and Methods

Literature review

We searched MEDLINE, EMBASE, and COCHRANE using the terms "DADA2" OR "ADA2" OR "CECR1" OR "deficiency of adenosine deaminase 2" OR "adenosine deaminase 2 deficiency" by Google Chrome (Google LLC. 90.0). The original searches were limited to English literature and dated from 2014 to February 28th, 2020. A manual search was performed from March 1st, 2020, to the December 31th 2020. Publications of other diseases, basic studies on the ADA2 protein/gene, and reviews without individual cases were excluded. One unreported case from our center was included.

Structured demographic data, symptoms, laboratory results, imaging, pathology, treatments, and prognosis were collected. Missing data were defaulted as none. Cases reported in different publications were included only once, and clinical data were implemented from duplicated reports. The inclusion criteria were cases that contained individual phenotypes and genotypes. The exclusion criteria were cases that were only suspected of the disease, died before genotype confirmation, and had no description of treatments or outcomes.

Definitions and subgroups

The age of disease onset was recorded according to the literature report. Asymptomatic patients who were diagnosed during screening had disease onset age as the age of their diagnosis. Patients with onset age of less than 18 years old were grouped into pediatric group while patients with onset age of 18 years or more were grouped into adult group. Positive family history was defined as there are siblings or other family members being affected. Genotypes of all patients were reviewed. Patients carrying only missense mutations were classified into the missense mutation group, while patients carrying at least one null mutation, including nonsense mutations, deletions, insertion-deletions, and frameshift mutations, were classified into the null mutation group. ADA2% refers to the ratio of the tested ADA2 level to the lower limit of normal range according to the literature report.

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All phenotypes were collected according to the reports and were classified into three domains: vasculitic manifestations, hematological abnormalities, and immunodeficiency (5). Vasculitic manifestations included constitutional symptoms, neurological events, skin lesions, organ involvement, peripheral vasculopathy and increased acute phase proteins (APRs). Peripheral vasculopathy referred to imaging evidenced peripheral vascular stenosis or aneurysm, episodes of organ infarction or biopsy confirmed vasculitis. Neurological events included cerebrovascular events, peripheral neuropathy, cognitive abnormalities, visual abnormalities and hearing abnormalities. Organ involvement referred to musculoskeletal disease, cardiovascular disease (hypertension, cardiomyopathy, pulmonary hypertension), intestinal disease (hepatosplenomegaly, gastrointestinal bleeding, portal hypertension, and inflammatory bowel disease), renal disease (urine abnormalities, nephritis, renal biopsy confirmed glomerular nephritis), testicular disease, and others. The numbers of involved organs were summed (No. organ). All the above symptoms were recorded as their original paper reported. APRs included C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum tumor necrosis factor α (TNFα), interleukin (IL)-6 (IL-6), IL-8, and IL-10. Increased APRs were defined as the reported APRs level higher than their normal range or the paper reported them as increased. Hematological abnormalities included anemia, leukopenia, thrombocytopenia, and pancytopenia as the paper reported. Bone marrow hypoplasia was defined as bone marrow failure, pure red cell anemia, Diamond-Blackfan anemia, and other types of decreased bone marrow proliferation. Immunodeficiency was defined as decreased immunoglobulin levels and/or B cells. Recurrent infections were defined as documented infections before treatment.

Controlled was defined as free from symptoms after treatment, while uncontrolled was defined as sustained active disease during treatment or early relapses during treatment tapering. Effective treatment was defined as the disease responded to the treatment. Ineffective treatment was defined as the disease not responded to the treatment.

Statistical analysis

Continuous variables are described as the median (range) and were compared with the

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Wilcoxon rank sum test. Counting variables are reported as numbers (frequencies) and were compared with the chi-square test. Single- and multivariate logistic regressions were performed to evaluate factors associated with well-controlled disease. STATA (14.0, Texas USA) was used as a statistical tool, and 2-sided *P*-value of less than 0.05 was designated as significant.

Results

Demographic data

A total of 274 cases were extracted from the literature review (see Supplementary Table E1). Thirty-four cases were excluded because of no reporting of disease outcome. (Fig.1). Thus, together with two patients from our center, a total of 242 patients were enrolled in this study, with 118 (48.8%) males and 124 (51.2%) females. The median age of disease onset was 4 years (range 0-59 year). 58 (24.0%) reported history of consanguinity and 123 (50.8%) reported positive family history. 17 patients had no or mild symptoms requiring no treatment. Compared to patients requiring active treatment, patients requiring no treatment had higher age of disease onset (median 7.5 (range 5-18) years of age vs. 4 (0-59) years of age, p=0.047), were more likely to be in the adult group (40% vs. 5.4%, p<0.001) and higher rates of positive family history (94.1% vs. 47.6%, p<0.001); while the two groups had similar rates of having null mutations (23.5% vs. 27.6%) and history of consanguinity (33.3% vs. 23.8%). (Table 1)

Among the remaining 225 patients received active treatment, vasculitic manifestations (215/225, 95.6%) were the most common symptoms. Increased APRs were reported in 96 patients (42.7%), which included CRP (64.6%, 62/96), ESR (44.8%, 43/96), TNFα (4/96, 4.2%), IL-6 (5/96, 5.2%), IL-8 (2/96, 2.1%), and IL-10 (4/96, 4.2%). Hematological symptoms were reported in 135 patients (60.0%), including 27 patients reported pancytopenia. Among patients with hematological symptoms, 63 patients recorded bone marrow aspiration, and 49 of whom reported bone marrow hypoplasia. Immunodeficiency was reported in 107 patients (47.6%). A total of 53 patients (23.6%) reported recurrent infections before treatment. Pathogens included bacterial, fungal, viral and mycobacterial infections. The detailed clinical features of these patients are shown in Table 2.

Treatment

Among the 225 patients requiring active treatment, the majority received more than one treatment. Generally, a total of 131 (57.9%) patients had received TNFi, including etanercept used in 59.5% (78/131), adalimumab in 22.1% (29/131), and infliximab in 12.2% (16/131), and 6.1% (8/131) did not report the type of TNFi. Generally, the effective rate of all TNFi users were 78.6% (103/131). High APRs, the number of vasculitic manifestations, hematological symptoms and recurrent infections were associated with TNFi effectiveness (Table 3).

In crude logistic analysis, high APRs and the number of vasculitic manifestations and high APRs were positively associated with TNFi effectiveness, OR=9.07 (95%CI 2.932-28.08, *P*-value<0.001) and 1.65 (95%CI 1.216-2.237, *P*-value 0.001), respectively; while hematological involvement and recurrent infections were associated with poor TNFi effectiveness, OR=0.35 (95%CI 0.141-0.864, *P*-value 0.023) and 0.24 (95%CI 0.098-0.615, *P*-value 0.003), respectively. In multivariate analysis, high APRs and hematological involvement remained independently associated with TNFi effectiveness, OR=9.62 (95%CI 2.313-40.0, *P*-value 0.002) and 0.21 (95%CI 0.070-0.661, *P*-value 0.007). Furthermore, in patients with bone marrow hypoplasia, TNFi effectiveness was 29.4% (5/17).

In subgroup analysis, patients in different age groups (78.5% vs. 80.0%, *P*-value 0.913) and mutation groups (81.4% vs. 70.6%, p=0.227) showed similar effective rate. To exclude the confounding effect from steroids, the calculated TNFi effectiveness after excluding the 25 patients who were taking both steroids and TNFi at outcome assessment were 79.2% (84/106). In crude and multivariate logistic analysis after excluding those 25 patients, increased APRs and hematological involvement remained independently associated with TNRi effectiveness, OR=10.39 (95%CI 2.088-51.74, *P*-value 0.004) and 0.15 (95%CI 0.038-0.561, *P*-value 0.005), respectively. (Table 3)

Steroids were the most commonly used medicine before the diagnosis of DADA2. A total of 164 patients had received steroids during their disease courses. 36 (22.0%) patients showed control of disease and successful tapering. 26 (15.9%) patients were dependent on large steroid doses and 13 (7.9%) patients had disease relapse after steroid tapering. 21 (12.8%) and 67

(40.9%) showed partial or no effectiveness in controlling the disease activity, respectively. 47 (28.6%) patients had to switch to TNFi due to ineffective or dependent use of steroids.

Traditional immunosuppressive agents and anti-inflammatory agents were reported in few cases with varying effectiveness (Table 4). Other biologic agents, including tocilizumab, IL-1 antagonist, and rituximab were reported in 10, 11 and 12 patients, respectively, and the effective rate were 20% (2/10), 9.1%(1/11) and 8.3% (1/12), respectively. Fresh frozen plasma (FFP) was used in 12 patients for presumed active ADA2 supplementation, while only one achieved temporal disease control for 8 months before he had another relapse [15]. Intravenous immunoglobulin (IVIG) was used mostly for immunoglobulin supplementation, only one patient reached disease control with large-dose IVIG [19]. 24 (10.7%) patients received hematopoietic stem cell transplantation (HSCT), 22 patients were due to uncontrolled hematological abnormalities. One patient received HSCT due to recurrent transient ischemic attack and hypoimmunoglobulinemia [20] and another patient received HSCT due to refractory cerebrovascular event [21]. 21 patients achieved restoration of immunity and control of systemic inflammation, while 2 patients died after HSCT and one still had hematological abnormality [22].

In addition, 28 (12.6%) patients received aspirin, 9 (4.0%) received anticoagulants, and 6 (2.7%) received both. 19/43 (44.2%) patients had refractory stroke and 5/43 (11.6%) had hemorrhagic events after anti-platelet and/or anti-coagulation therapy. Of those received concurrent steroids, 14/16 (87.5%) patients had new ischemic events; while of those received concurrent TNFi, 4/15 (26.7%) had new ischemic events. Of the 5 patients had hemorrhagic events, 3 had concurrent steroids, and none had concurrent TNFi.

Disease control

Among the 255 patients received active treatment, 157 (69.8%) patients had reached disease control, and 68 (30.2%) patients were uncontrolled by the time of the report. A total of 21 patients died, 10 patients died due to fatal infections, 6 due to intracranial hemorrhage or intestinal perforation, 2 patients due to both vascular event and severe infection, 1 due to refractory hematological manifestations and 2 patients due to disease complications after

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disease activity control. 4 patients died before the diagnosis of DADA2.

The two groups had similar disease onset ages, ADA2% and frequencies of null mutations. Patients in the controlled group had significantly more vasculitic manifestations and organ involvement, higher rates of having increased APRs, constitutional symptoms and neurological symptoms, but less rate of having hematological abnormalities or recurrent infections. TNFi or HSCT treatment was more often reported in patients in the controlled group. (Table 2)

In crude analysis, the number of vasculitic manifestations, the number of organ inflammation, constitutional symptoms, neurological symptoms and increased APRs were associated with disease control while hematological manifestations and recurrent infections were associated with uncontrolled disease. Compared to those receiving neither steroid nor TNFi, TNFi with or without steroids were associated with disease control, but not steroids alone. HSCT also improved the odds of disease control. (Table 5)

In multivariate analysis of all the above clinical factors and treatment protocols, constitutional symptoms, neurological events and increased APR remained independently associated with disease control, OR=7.17 (95%CI 2.398-21.47, *P*-value 0.007), 4.56 (95%CI 1.587-13.10, *P*-value 0.005) and 5.00 (95%CI 1.668-15.00, *P*-value 0.004), respectively; while the number of vasculitic manifestations and recurrent infections were independently associated with poor disease control, OR=0.49 (95%CI 0.287-0.822, *P*-value 0.007) and 0.35 (95%CI 0.144-0.869, *P*-value 0.024), respectively. Meanwhile, TNFi with (OR=8.81, 95%CI 2.144-36.19, P value 0.003) or without concurrent steroid usage (OR=10.1, 95%CI 3.780-26.98, P-value <0.001), as well as HSCT (OR=29.2, 95%CI 6.177-138.0, P-value <0.001) were associated with disease control while steroid usage (OR=0.94, 95%CI 0.333-2.647, P-value 0.904) was not associated with disease control. (Table 5)

Discussion

Since DADA2 is a rare disease with diverse clinical presentations and high mortality without appropriate treatment, its appropriate therapy could be a great challenge. Although some consensus have raised in recent years, the effectiveness of therapies vary, and studies focusing on factors for treatment outcome are scarce. To determine the factors associated with good response to therapies, we summarized the treatments and outcomes in the present review.

Clinical profile might vary due to genetic background and the age of disease onset [18]. In the present study, although patients requiring no treatment tend to be older at disease onset age, we found that neither disease onset age nor genetic background impacted the disease outcome in those requiring treatment. Thus, other factors for clinical outcome were investigated in this review.

Among patients receiving active treatment, having recurrent infections and more vasculitic manifestations were associated with uncontrolled disease. This is consistent with the finding that recurrent infections and severe vascular events were the main causes of death. Therefore, recurrent infections and severe vascular manifestations warrant intensive treatment as they pose a high risk for poor disease outcome. It might be noted that recurrent infections in patients with inborn errors are multifactorial. Clinical presentations are highly heterogeneous and managements require multidisciplinary cooperation [23].

On the contrary, increased APRs, having constitutional symptoms and neurological symptoms were independently associated with controlled disease. Increased APR and constitutional symptoms are signs for systemic inflammation, while neurological symptoms are signs of vasculitis. Rama et al [24], after evaluating 13 genetically confirmed DADA2 patients along with 53 clinical suspected patients, found that fever with neurological symptoms along with either increased CRP or cutaneous manifestations would highly predict the disease, OR=13.9 (95%CI 1.86-172.87) and 17.72 (95%CI 1.53-955.70), respectively. Therefore, these symptoms would promote early diagnosis of the disease and therefore early intervention. Furthermore, Schnappauf et al [25] reported one patient who was asymptomatic but had increased APRs when diagnosed and then had an ischemic stroke ten months later. Therefore,

APRs might be used as a guide for treatment event in asymptomatic patients. Insalaco et al [26] suggested increased interferons as biomarkers for disease activity, but the APRs in the present study were mainly CRP and/or ESR, which are more readily available for most institutions and predominantly used in practice.

Recently, several studies have demonstrated the effectiveness of TNFis in controlling the disease [7, 27-29]. TNFi could reduce pediatric vasculitis activity score (PVAS) from 20/63 (IQR 13.0-25.8/63) to 2/63 (IQR 0-3.8/63) [30] and also the rate of stokes from 55 times per 2077 patient-months to 0 per 733 patient-months [31]. The present review found that the general effective rate of TNFis was 78.6%, and the use of TNFis, with or without steroids, is significantly associated with disease control. Thus, factors associating TNFi effectiveness are of clinical importance for forecasting the treatment response with this agent.

We found that increased APRs was associated with TNFi effectiveness, which might be due to the increased production of TNFα in both peripheral serum and affected tissues [2, 7]. Preliminary studies have reported TNFi treatment reduced tissue and serum inflammatory mediators such as IL-1β, IL-6 and TNF cytokines, and improved small vessel endothelial integrity [8]. On the contrary, we found that having hematological manifestations was associated with decreased effectiveness of TNFi. This finding was in consistent with previous observations that TNFi were ineffective in correcting hematological symptoms [22, 30], and patients with refractory hematological abnormalities were the top reason for having to resort to HSCT. Whereas, Ombrello et al [31] reported that 4 patients who were given TNFis had resolved anemia, and 5 had normalized hepatomegaly. We noticed that patients with bone marrow hypoplasia were more likely to fail TNFi treatment while patients with normal bone marrow might still respond to TNFi, which probably because systemic inflammation could also cause hematologic symptoms without affecting the bone marrow. Thus, we suggest that bone marrow aspiration should be conducted in patients with DADA2 with hematological abnormalities, for those with normal bone marrow, TNFi might still be effective while those with bone marrow hypoplasia might consider other treatment or even HSCT as TNFi treatment might not be effective.

Recently, a treatment guideline based on an international Delphi survey has suggested TNFi therapy as the first line therapy and potentially for indefinite use [32]. Long-term use of TNFi would raise the concern of antidrug antibody, which was reported in 20.8% of patients with rheuamtoid arthritis and 27.7% of patients with inflammatory bowel disease during the first 12 months of TNFi treatment [33]. On the other hand, Verboom et al [34] reported 8/9 patients with Behcet's disease receiving adalimumab for up to 5 years without generating antidrug antibodies. Thus, generation of antidrug antibody might be multifactorial. DADA2 is a rare autoinflammatory disease without antibody generation. Treatment with TNFi in this disease has been started recently. For that reason, the problem of antidrug antibody would require long-term surveilance. Immunogenicity is more common in patients treated with infliximab (a murine-human chimeric monoclonal antibody) than adalimumab (a fully human monoclonal antibody) and is a major cause of low anti-TNF drug level, infusion reactions, and non-remisision outcomes [35, 36].

In the present study, steroid usage was not associated with disease outcome, and the effectiveness of steroids were low. However, we found that steroids were effective at large dosages (0.5-2mg/kg/d). Thus, large dose steroids might be used for quick disease control during acute flares but could not be used for long-term disease control. Thalidomide, previously reported in only 8 cases showed good response (6/8, 75%), probably due to its anti-TNFi effect [7], though its side-effects should be paid attention to. HSCT is the only therapy that can cure the disease, restore serum ADA2 levels and reconstruct the immune system [20]. However, complications of HSCT, including early infections, graft-versus-host disease, and post-HSCT autoimmune phenomena (reviewed by Kendall et al [3]), have restricted its clinical application. Intriguingly, Carmona-Rivera et al [4] revealed that recombinant ADA2 could ameliorate NETosis which is one of the main pathophysiological changes in DADA2. Thus, further gene therapy aimed at reconstructing exogenous ADA2 might be promising.

Due to vascular events, antiplatelet and/or anticoagulation therapies are frequently used. Nevertheless, the present study indicated that they failed to prevent ischemic events even with concurrent steroids, and posed risks to hemorrhagic events. Given the high risk of both ischemic

and hemorrhagic events in this disease, the use of these agents is not recommended before disease control [32]. The application of antiplatelets and/or anticoagulants in patients after disease control might require further investigation.

This study bares some limitations. First, DADA2 is a rare disease, which makes large cohort studies or well-designed clinical trials difficult. Data from case reports might be incomplete. Such a literary review based on previous case reports possesses high heterogeneity and therefore systemic bias. Second, the actual treatments of previous real-world cases were complicated. Some patients had received more than one regimen, and their regimen changed constantly during their disease course, which made the investigation of individual medicine difficult. Finally, only qualitative analysis was performed, and we could not calculate the survival curve due to the heterogeneity of these case reports. Nevertheless, the results from this study might still shed some light on the clinical management and mechanical study of the disease.

In conclusion, we found that although the genetic background and disease onset age might vary the clinical profiles of a patient, neither of them was involved in disease outcome. Increased APRs was associated with better disease control and TNFi effectiveness, and could be used as a marker for monitoring disease activity. Constitutional symptoms and neurological symptoms could suggest early disease diagnosis and therefore early treatment; while recurrent infections and severe vascular events pose risks for uncontrolled disease and mortality. Treatment with TNFi was highly effective in controlling the systemic inflammation and vasculitic manifestation, but not the hematological abnormalities. Bone marrow aspiration might be useful for guiding the use of TNFi, as those with bone marrow hypoplasia might not respond to TNFi.

DADA2 is a multisystem disease requiring incorporation of multidisciplinary management more than just TNFi treatment. Clinical therapeutic strategies are complicated and individualized, depending on the time of diagnosis, complications during treatment, expert experience of the medical center, availability to the proper treatment and the economic considerations. Future bench and bedside studies might answer the remaining questions on

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DADA2, including the treatment duration, monitoring of TNFi and anti-TNFi antibodies, anticoagulation regimen and the feasibility of genetic therapies, while multidisciplinary management might be crucial for long-term disease control.

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Conflicts of interest

None

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Figure Legend

Fig.1 Flow chart of literary review process

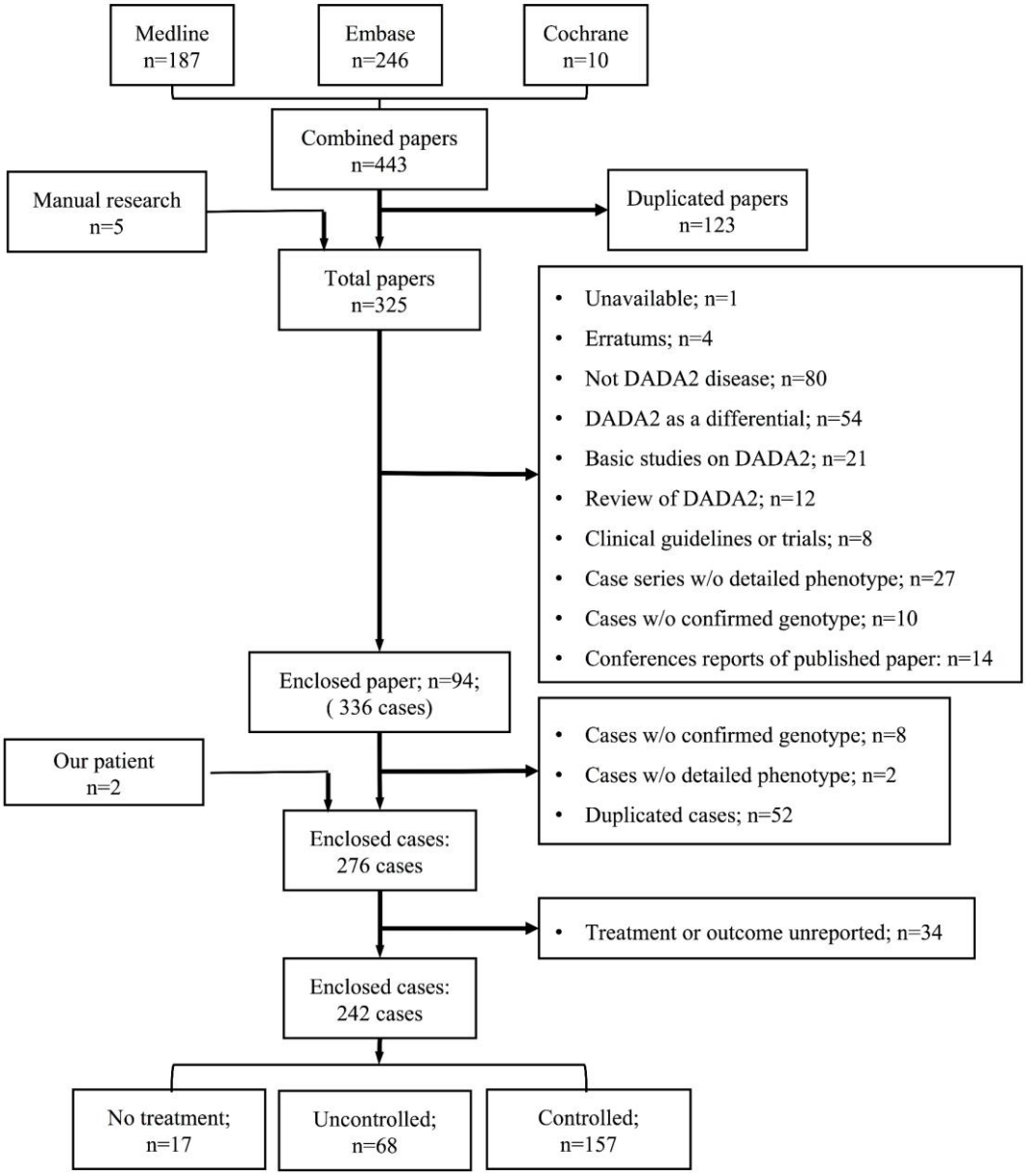

Fig. 1 Flow chart of literary review process

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Table 1 Geographic characteristics of DADA2 patients

	Total	Requiring no treatment	Requiring active treatment	P-value
No.	242	17	225	
Male (%)	118 (48.8)	9 (52.9)	109 (48.4)	0.804
Median age of onset (range)	4 (0-59)	7.5 (5-18)	4 (0-59)	0.047
Adult group (%)	18 (7.5)	6 (40.0)	12 (66.7)	<0.001
Consanguinity (%)	58 (24.4)	5 (33.3)	53 (23.8)	0.370
Family history (%)	123 (50.8)	16 (94.1)	107 (47.6)	<0.001
Null mutations (%)	66 (27.3%)	4 (23.5%)	62 (27.6%)	1.00
Median ADA2% (rang)	1.3% (0-63%)	1.3% (1%-63%)	1.28% (0-50%)	0.338

Table 2 Clinical characteristics of DADA2 patients requiring treatment

	Total	uncontrolled	controlled	*P*-value
No.	225	68	157	
Male (%)	109 (48.4)	31 (45.6)	78(49.7)	0.573
Median age of onset (range)	4 (0-59)	2.5 (0-59)	4 (0-44)	0.177
Consanguinity (%)	53 (23.6)	12 (17.1)	41 (26.5)	0.169
Family history (%)	107 (47.6)	34 (50.0)	73 (46.5)	0.629
Null mutations (%)	62 (27.6)	22 (32.4)	40 (25.5)	0.289
Median ADA2% (range)	1.28% (0-50%)	1.80% (0-17.4%)	0.8% (0-50%)	0.063
Vasculitic manifestation (%)	215 (95.6)	63 (92.6)	152 (96.8)	0.164
No. vasculitic manifestation (range)	4 (0-6)	3 (0-6)	4 (0-6)	0.001*
Increased APRs (%)	96 (42.7)	19 (27.9)	77 (49.0)	0.003*
Constitutional (%)	128 (56.9)	30 (44.1)	98 (62.4)	0.011*
Dermatological (%)	170 (75.6)	47 (69.1)	123 (78.3)	0.139
Organ inflammation (%)	175 (77.8)	52 (76.5)	123 (78.3)	0.756
No. organ inflammation (SD)	1 (0-5)	1 (0-3)	1 (0-5)	0.048*
Neurological involvement (%)	138 (61.3)	30 (44.1)	108 (68.8)	<0.001*
Cerebrovascular events (%)	87 (38.8)	15 (22.1)	72 (46.2)	0.001*
Hematology (%)	135 (60)	51 (75.0)	84 (53.5)	0.003*
Pancytopenia (%)	27 (12.0)	14 (21.0)	13 (8.3)	0.008*
Immunodeficiency (%)	107 (47.6)	35 (51.5)	72 (45.9)	0.470
Recurrent infections (%)	53 (23.6)	25 (36.8)	28 (17.8)	0.002*
Treatment				
Others	40 (17.8)	25 (35.7)	15 (9.7)	
TNFi based (%)	99 (44.0)	15 (20.6)	85 (54.1)	
Steroid based (%)	37 (16.4)	20 (29.4)	17 (10.8)	
TNFi+steroid (%)	25 (11.1)	6 (8.8)	19 (12.1)	
HSCT (%)	24 (10.7)	3 (4.4)	21 (13.4)	

*: *P*-value <0.05;

No. vasculitic manifestation: cumulative number of the following symptoms: high acute phase reactant (APR), constitutional symptoms, dermatological symptoms, organ inflammation, neurological symptoms, peripheral vasculopathy

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No. organ inflammation: cumulative number of the following organ involvement: cardiopulmonary events, intestinal inflammation, nephritis or urine abnormality, musculoskeletal involvement

ADA2%: the ratio of tested ADA2 level to the lower limit of normal range; APRs: Acute phase reactants, including C-reactive protein, Erythrocyte sedimentation rate, IL-6, IL-8, IL-10. TNFi: TNFα inhibitors; HSCT: hematopoietic stem cell transplantation.

Table 3 Factors associated with good response to TNFα inhibitors therapy

Factors	total	Crude			Multivariate analysis		
		ineffective	effective	*P*-value	OR	95%CI	*P*-value
Subgroup 1							
Number	131	28	103				
High APRs	66 (50.4%)	4 (14.3%)	62 (60.2%)	<0.001	9.62*	2.313-40.00	0.002
No. vasculitic manifestations	4 (0-6)	3 (0-6)	4 (1-6)	0.005	1.19	0.821-1.736	0.352
Hematology	68 (51.9%)	20 (71.4%)	48 (46.6%)	0.032	0.21*	0.070-0.661	0.007
Recurrent infections	28 (21.4%)	12 (42.9%)	16 (15.5%)	0.004	0.98	0.302-3.175	0.972
Subgroup 2							
Number	106	22	84				
High APRs	51 (48.1%)	4 (18.2%)	47 (56.0%)	0.002	10.39*	2.088-51.74	0.004
No. vasculitic manifestations	4 (0-6)	3.5 (0-6)	4 (1-6)	0.056	1.10	0.746-1.648	0.639
Hematology	50 (47.2%)	15 (68.2%)	35 (41.7%)	0.032	0.15*	0.039-0.561	0.005
Recurrent infections	21 (19.8%)	7 (16.7%)	14 (31.8%)	0.136	2.04	0.479-8.708	0.334

* *P*-value <0.05 in multivariate analysis; APRs: acute phase reactants

Subgroup 1: all patients received TNFα inhibitors; Subgroup 2: to avoid confounding effects from steroids, patients with both TNFα inhibitors and steroid at outcome assessment were excluded.

No. vasculitic manifestations: cumulative number of the following symptoms: high acute phase reactant (APR), constitutional symptoms, dermatological symptoms, organ inflammation, neurological symptoms, peripheral vasculitis or vascular events.

Table 4 Treatments used in DADA2 patients except steroids and TNFα inhibitors

Treatments	Number of usage (%)	Number of effective control by monotherapy(%)	Number of effective control used with steroid &/or TNFi
Cyclophosphamide	49 (21.8)	0	11 (22.4%)
Mycophenolate mofetil	28 (12.4)	3 (10.7%)	4 (14.3%)
Methotrexate	25 (11.1)	2 (8.0%)	6 (24.0%)
Cyclosporine	13 (5.8)	1 (7.7)	0
Azathioprine	47 (20.9)	5 (10.6)	14 (29.8%)
Colchicine	25 (11.1%)	3 (12.0)*	0
Thalidomide	8 (3.5%)	6 (75%)	0
Sirolimus	6 (2.7%)	1 (16.7)	0
Tocilizumab	10 (4.4%)	2 (20.0%)	1 (10.0%)
IL-1 antagonists	11 (4.9)	1 (9.1%)	0
Rituximab	13 (5.8)	1 (7.7%)	3 (23.1%)
Intravenous immunoglobulin	32 (14.2%)**	1 (3.1)	0
Fresh frozen plasma	12 (5.3)	1 (8.3%)***	0

* only effective in treating cutaneous manifestations; ** 17 patients received intravenous immunoglobulin as supplementary to low immunoglobulin level. *** the patient remained stable with monthly fresh frozen plasma of 10ml/kg, but had constitutional symptoms 8 months later.

Table 5 Multivariate analysis of factors predicting disease control

Factors	Crude			Multivariate analysis		
	OR	95%CI	*P*-value	OR	95%CI	*P*-value
No. vasculitic manifestation	1.37	1.143-1.640	0.001	0.49*	0.287-0.822	0.007
Constitutional symptoms	2.10	1.181-3.748	0.012	7.17*	2.398-21.47	<0.001
No. organ inflammation	1.37	1.010-1.860	0.043	1.44	0.930-2.222	0.103
Neurological involvement	2.79	1.555-5.015	0.001	4.56*	1.587-13.10	0.005
High APRs	2.48	1.342-4.592	0.004	5.00*	1.668-15.00	0.004
Hematology	0.38	0.204-0.722	0.003	0.46	0.201-1.058	0.068
Recurrent infections	0.37	0.197-0.708	0.003	0.35*	0.144-0.869	0.024
Treatment						
others	1			1		
TNFi based	10.12	4.308-23.77	<0.001	10.1*	3.780-26.98	<0.001
Steroid based	1.42	0.570-3.519	0.453	0.94	0.333-2.647	0.904
TNFi+steroid	5.28	1.724-16.16	0.004	8.81*	2.144-36.19	0.003
HSCT	11.67	2.969-45.85	<0.001	29.2*	6.177-138.0	<0.001

* *P*-value <0.05 in multivariate analysis;

No. vasculitic manifestation: cumulative number of the following symptoms: high acute phase reactants (APRs), constitutional symptoms, dermatological symptoms, organ inflammation, neurological symptoms, peripheral vasculopathy.

No. organ inflammation: cumulative number of the following organ involvement: cardiopulmonary events, intestinal inflammation, nephritis or urine abnormality, musculoskeletal involvement,

APRs: Acute phase reactants; TNFi: TNFα inhibitors; HSCT: hematopoietic stem cell transplantation