Clinical and Experimental Immunology, 2023, **XX**, 1–4
https://doi.org/10.1093/cei/uxad122
Advance access publication 14 November 2023
Letter to the Editor

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Gender-affirming hormone therapy and autoimmunity: new insights from a 3-year follow-up study

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Keywords: transgender, autoimmune diseases, autoantibodies, gender affirming hormone therapy, gender medicine

Abbreviations: aCL: anti-cardiolipin; AFAB: assigned female at birth; AMAB: assigned male at birth; ANA: anti-nuclear antibodies; CPA: analogs or cyproterone acetate; ENA: extractable nuclear antigen; GAHT: gender-affirming hormone therapy; TGD: transgender and gender diverse; β2-GPI: anti-β2-glycoprotein I.

Dear Editor,

Transgender and gender diverse (TGD) people represent a broad spectrum of individuals whose gender identities and/or gender expressions are not what is typically expected for the sex assigned at birth [1]. The size of adult TGD population ranges, according to the most recent population-based studies, from 0.3% to 4.5% across the world [1]. TGD persons may require gender-affirming hormone therapy (GAHT) to achieve body changes consistent with their gender identity [1]. Feminizing and de-masculinizing GAHT typically consists of oral or transdermal estradiol and androgen-lowering medications [e.g. in Europe usually gonadotropin-releasing hormone (GnRH) analogs or cyproterone acetate (CPA)], whereas masculinizing GAHT typically consists of testosterone [1, 2]. GAHT for both assigned female at birth (AFAB) and assigned male at birth (AMAB) TGD people confers many of the same risks (e.g. thromboembolism, cardiovascular risk) observed with sex hormone replacement therapy in cisgender persons and careful monitoring and screening are required to reduce adverse events [1–3].

There are well-described sex-based differences in the functioning of the immune system [4–6]. In general, AFAB people have a more easily activated immune system associated with an increased prevalence of autoimmune diseases and adverse events following vaccinations. Conversely, AMAB people have a higher threshold for immune activation, and are more prone to certain infectious diseases. This sex bias in the immune responses is due to both genetic and hormonal factors [4–6]. As far as genetic factors are concerned, the role of the X chromosome has been observed to be fundamental [4–7]. However, the role played by sex hormones appears also to be crucial, with estrogens being potent immunostimulators and androgens playing an immunosuppressive role [4–6]. Despite this, potential immunological effects of GAHT in TGD people remains an unexplored area of research [8] and a small number of case reports describe the onset of autoimmune diseases, mainly systemic lupus erythematosus but also systemic sclerosis, rheumatoid arthritis, and other rheumatic diseases, in TGD people undertaking this therapy [9, 10]. Interestingly, a recent cross-sectional study showed that TGD people had higher rates of anti-nuclear antibodies (ANA), which represent a marker of self-reactivity across multiple autoimmune diseases, compared with the general population [11].

Hence, further research is needed to increase our knowledge into the immunological impact of GAHT with the final aim to ensure a more appropriate and personalized management of this therapy in TGD people. In this regard, we carried out a pilot study to evaluate the effects of GAHT on immunological biomarkers in TGD individuals undertaking this therapy during a 3-year follow-up. In particular, ANA and extractable nuclear antigen (ENA) antibodies, widely used in clinical practice and included in the diagnostic criteria for different autoimmune diseases [12] and anti-cardiolipin (aCL) and anti-β2-glycoprotein I (β2-GPI) antibodies, associated with the occurrence of arterial and venous thrombosis in antiphospholipid syndrome [13, 14], were studied. To this aim, serum samples from a consecutive series of 54 TGD people, who referred to the University Florence Hospital, were collected before (t0) and after 12, 24, and 36 months of GAHT (t12, t24, and t36, respectively). The 53.7%

Received 15 September 2023; Revised 11 October 2023; Accepted for publication

($n = 29$) of TGD people were AFAB (mean age 26 ± 8 years) and the 46.3% ($n = 25$) were AMAB (mean age 27 ± 11 years). Enrolled subjects were over the age of 18 years, had a diagnosis of gender dysphoria [5] plus requested GAHT in order to obtain a full feminization and demasculinization (AMAB people) and a full masculinization (AFAB people), and had never taken GAHT before the start of the study. Sociodemographic, laboratory (including routine blood tests, coagulation parameters, testosterone and 17β-estradiol levels), and clinical information was collected at study entry and again during follow-up. GAHT in AFAB people consisted of testosterone undecaoate 1000 mg i.m. (the second injection repeated after 6 weeks, then after 12 weeks) or transdermal testosterone gel (50–60 mg/day). In AMAB people, estrogen treatment consisted of oral estradiol valerate (2–6 mg/day) or estradiol patch (25–50 μg/day) or estradiol hemihydrate or valerate gel (1–3 mg/day). Additionally, AMAB transgender persons started oral anti-androgen treatment (CPA, 50–100 mg/day). Despite different treatment regimens, target testosterone levels were maintained between 320 and 1000 ng/dl in AFAB people with the aim to abstain a full virilization and estradiol between 100 and 200 pg/ml with testosterone levels < 50 ng/dL in AMAB people with the goal to obtain a full feminization and de-masculinization according to current guidelines [1, 4]. ANA were tested by indirect immunofluorescence test (IF) on HEp-2 cells (ZENIT RA ANA; A. Menarini Diagnostics Ltd, Florence, Italy). ENA, aCL and anti-β2-GPI antibodies were measured using chemiluminescent immunoassay (Bio-Flash, Werfen, San Diego, USA). The study was conducted in compliance with the Helsinki declaration and approved by the institution's ethics committee (CEAVC Em. 2021-502); participants gave written informed consent.

The results obtained are summarized in Table 1. At t0, two out of 29 AFAB TGD people (aged 39 and 21 years) were

Table 1. Autoantibody prevalence in TGD subjects before and during GAHT

Autoantibodies	t0				Follow-up			
	AFAB		AMAB		AFAB		AMAB	
	N	%	*N*	%	*N*	%	*N*	%
Anti-nuclear antibodies (ANA)	1/29	3.5	0/25	0.0	2/29	6.9	0/25	0.0
Anti-extractable nuclear antigen antibodies (ENA antibodies)	0/29	0.0	0/25	0.0	0/29	0.0	0/25	0.0
Anti-cardiolipin antibodies (aCL IgM)	3/29	10.3	0/25	0.0	2/29	6.9	1/25	4.0
Anti-cardiolipin antibodies (aCL IgG)	0/29	0.0	0/25	0.0	0/29	0.0	0/25	0.0
Anti-β2-glycoprotein I antibodies (β2-GPI IgM)	1/29	3.5	0/25	0.0	0/29	0.0	0/25	0.0
Anti-β2-glycoprotein I antibodies (β2-GPI IgG)	0/29	0.0	0/25	0.0	0/29	0.0	0/25	0.0

Column "t0" indicates the number (and relative percentages) of AFAB and AMAB TGD subjects positive for autoantibodies before GAHT. The "follow-up" column summarizes data collected after 12, 24, and 36 months of GAHT. Data are reported as absolute numbers (*N*) and percentages (%). AFAB, assigned female at birth; AMAB, assigned male at birth; GAHT, gender-affirming hormone therapy.

Table 2. Autoantibody levels in AFAB and AMAB TGD subjects positive for autoantibodies

AFAB subjects

	aCL IgM (MPL/ml)		aCL IgG (GPL/ml)		anti-β2-GPI IgM (U/ml)		anti-β2-GPI IgG (U/ml)		ENA (U/ml)		ANA	
	t0	Follow-up	t0	Follow-up	t0	Follow-up	t0	Follow-up	t0	Follow-up	t0	Follow-up
1	**18.2**	**19.4**	0.0	0.0	3.5	3.3	0.0	0.0	0.0	0.2	Neg	Neg
2	**19.3**	9.9	0.0	0.0	**20.3**	10.8	0.0	0.0	0.7	0.8	**1:320**	**1:320**
3	**15**	**15**	0.0	0.0	1.3	3.4	0.0	0.0	0.6	0.8	Neg	Neg
4	6.3	5	0.0	0.0	1.6	3.9	0.0	0.0	0.1	0.1	Neg	**1:160**

AMAB subjects

	aCL IgM (MPL/ml)		aCL IgG (GPL/ml)		anti-β2-GPI IgM (U/ml)		anti-β2-GPI IgG (U/ml)		ENA (U/ml)		ANA	
	t0	Follow-up	t0	Follow-up	t0	Follow-up	t0	Follow-up	t0	Follow-up	t0	Follow-up
1	3.9	**16.6**	0.0	0.0	0.3	0.4	0.3	0.0	0.4	0.6	Neg	Neg

Column "t0" indicates data collected before GAHT. The "follow-up" column summarizes data collected after 12, 24, and 36 months of GAHT. AFAB, assigned female at birth; AMAB, assigned male at birth; GAHT, gender-affirming hormone therapy; aCL, anti-cardiolipin antibodies; anti-β2-GPI, anti-β2-glycoprotein I antibodies; ANA, anti-nuclear antibodies; ENA, anti-extractable nuclear antigen antibodies. Ct-off values: aCL IgM: 15 MPL/ml; aCL IgG: 15 GPL/ml; anti-β2-GPI IgM: 15 U/ml; anti-β2-GPI IgG: 15 U/ml; ENA: 1 U/ml; ANA: 1:80.

positive for IgM aCL antibodies (18.2 and 15 MPL/ml, respectively) and one (aged 41) was positive for IgM aCL (19.3 MPL/ml), IgM anti-β2-GPI (20.3 AU/ml) antibodies, and ANA (1:320, homogeneous pattern). In the first two subjects, there were no changes during GAHT in their autoantibody profiles. In the third subject, IgM aCL and IgM anti-β2-GPI antibodies negativized at t36, while ANA remained unchanged throughout the follow-up. In addition, one AFAB subject (aged 44 years) became positive for ANA (1:160, homogeneous pattern) since t24. Among AMAB TGD subjects, none out of 25 subjects was positive for antibodies tested at t0; one AMAB subject (aged 19 years) became positive for IgM aCL antibodies (16.6 MPL/ml) at t36. In summary, of the five cases described above, three AFAB subjects were positive for autoantibodies tested at t0 and showed no increase in their serum levels during follow-up, one AFAB subject positivized at t24, showing no increase in antibody serum values in the next follow-up, and one AMAB subject positivized at t36 (Table 2).

To note, none of these subjects had evidence of rheumatic conditions but also of other pathologies, such as tumors and infections that may associate with autoantibody positivity [13, 14] (data not shown). In conclusion, in this study, the first one in our knowledge that prospectively evaluated the presence of autoantibodies in TGD people undergoing GAHT, we observed that 3 years of this therapy does not increase the risk of autoimmune diseases, at least those that are characterized by the presence of antibodies tested in this study. However, a correlation between the occurrence of autoantibodies (or modification of their titers) and GAHT cannot be completely ruled out, due to the small numbers of subjects tested. To note, it is important to remember that ANA may precede the development of autoimmune diseases by years and that they are frequently found in a proportion of general population in which their presence, especially at high titers, is significantly associated with the risk of developing a connective tissue disease [12]. On the other hand, regarding aCL antibodies, reports from different laboratories show an apparent large number of normal individuals with low to moderate titers of these autoantibodies of the IgM isotype without any apparent clinical manifestation [13]. In addition, evidence for an association between anti-β2-GPI antibodies of the IgM isotype and antiphospholipid syndrome is weak [15]. Thus, it is critical to carry out large-scale longitudinal prospective studies enrolling TGD people to definitively exclude that the appearance of autoantibodies in TGD persons undertaking GAHT translates into autoimmune diseases. Moreover, in a broader perspective, these studies could enhance our understanding of the role of sex hormones in autoimmune diseases, independently by the role played by sex chromosomes.

Acknowledgements

We thank Alessandro Lisci for his precious technical assistance.

Ethical approval

The study was conducted in compliance with the Helsinki declaration and approved by the institution's ethics committee (CEAVC Em. 2021-502, Florence University Hospital, Florence, Italy).

Conflict of interests

The authors declare no financial or commercial conflicts of interest.

Funding

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

M.M. analyzed data and wrote the manuscript; G.R., M.T.P., A.C., A.L. performed the laboratory measurements and analyzed the data; A.D.F., C.C., S.D.'A. recruited and characterized patients and contributed to data interpretation; L.V. and M.S. contributed to data interpretation and manuscript revision; E.O. contributed to the conception and design of the work and manuscript revision; M.P. contributed to the conception and design of the work, manuscript revision and supervision. All authors contributed to the article and approved the manuscript.

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