TRANSLATIONAL SCIENCE

B cell depletion impairs vaccination-induced CD8⁺ T cell responses in a type I interferon-dependent manner

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ABSTRACT

Objectives The monoclonal anti-CD20 antibody rituximab is frequently applied in the treatment of lymphoma as well as autoimmune diseases and confers efficient depletion of recirculating B cells. Correspondingly, B cell-depleted patients barely mount de novo antibody responses during infections or vaccinations. Therefore, efficient immune responses of B cell-depleted patients largely depend on protective T cell responses.

Methods CD8⁺ T cell expansion was studied in rituximab-treated rheumatoid arthritis (RA) patients and B cell-deficient mice on vaccination/infection with different vaccines/pathogens.

Results Rituximab-treated RA patients vaccinated with Influvac showed reduced expansion of influenza-specific CD8⁺ T cells when compared with healthy controls. Moreover, B cell-deficient JHT mice infected with mouse-adapted Influenza or modified vaccinia virus Ankara showed less vigorous expansion of virus-specific CD8⁺ T cells than wild type mice. Of note, JHT mice do not have an intrinsic impairment of CD8⁺ T cell expansion, since infection with vaccinia virus induced similar T cell expansion in JHT and wild type mice. Direct type I interferon receptor signalling of B cells was necessary to induce several chemokines in B cells and to support T cell help by enhancing the expression of MHC-I.

Conclusions Depending on the stimulus, B cells can modulate CD8⁺ T cell responses. Thus, B cell depletion causes a deficiency of de novo antibody responses and affects the efficacy of cellular response including cytotoxic T cells. The choice of the appropriate vaccine to vaccinate B cell-depleted patients has to be re-evaluated in order to efficiently induce protective CD8⁺ T cell responses.

INTRODUCTION

Antibody responses play a key role in mediating protection against severe infections and the efficacy of the majority of currently available vaccines relies on the induction of long-lasting antibody responses. In particular during the current SARS-CoV-2 pandemic, it is discussed to which extent antibody and T cell responses contribute to protection. In some convalescent patients, rapidly decreasing antibody titres were observed. The question arose, whether such patients are still protected from SARS-CoV-2 reinfection by long-lasting T cell memory.

B cell depletion using the anti-CD20 antibody rituximab is an effective treatment of lymphoproliferative diseases such as non-Hodgkin’s lymphomas, various autoimmune diseases, including immune thrombocytopenia (ITP), rheumatoid arthritis (RA), anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, systemic lupus erythematosus, multiple sclerosis, and prevents graft failure after some solid organ transplantations. Since B cell depletion massively reduces the formation of SARS-CoV-2-specific antibodies, it is intensively discussed whether B cell depleting therapy with rituximab and Ocrelizumab should be postponed until SARS-CoV-2 vaccination has been performed. In the absence of antibody responses, CD8⁺ cytotoxic T cells take over important functions in protection against pathogens. For B cell-depleted patients it is therefore of utmost importance to mount functional CD8⁺ T cell responses upon vaccination.
Rheumatoid arthritis

Recently, it became evident that B cell depletion influences CD8+ T cell responses. In a murine model of ITP, rituximab treatment inhibited splenic CD8+ T cell proliferation and thus protected against T cell-mediated autoimmune thrombocytopenia. Furthermore, it was reported that B cells promote survival of intra-islet CD8+ T cells in NOD mice and that B cell deficiency significantly delayed diabetes development. B cells also play a specific role in modulating the contraction of CD8+ T cell responses following immunisation with Listeria monocytogenes and in establishing efficient CD8+ T cell memory. Furthermore, B cells were required to prevent virus-specific CD8+ T cell memory exhaustion upon lymphocytic choriomeningitis virus infection.

Whether B cells support T cell responses by direct cell-cell contact or via cytokine and chemokine expression is still largely unclear. A CXCR5+ subset of CD8+ T cells was shown to constitute early effector cells that migrate into B cell follicles and thus might be able to directly interact with B cells. Several chemokines and cytokines such as type I interferon (IFN-I) were shown to orchestrate lymphocyte responses locally or via systemic inflammatory signals. In addition to direct anti-viral function, IFN-I directly triggers the IFN-I receptor (IFNAR) of CD8+ T cells to promote their expansion.

IFN-I are potent antiviral cytokines that are induced early upon various infections and thus are targeted by many viral evasion strategies. The poxvirus strains vaccinia virus (VACV) and modified vaccinia virus Ankara (MVA) are relevant vaccine models to study vaccination in vivo. In contrast to its parental strain VACV, MVA lost several IFN-I inhibitors during passaging on chicken embryo fibroblasts and therefore efficiently induces serum IFN-I responses in mice.

Here, we studied the impact of B cell depletion on CD8+ T cell expansion during immunisation with different viruses. We found massively reduced CD8+ T cell responses in B cell-depleted RA patients upon influenza vaccination. CD8+ T cell expansion was also strongly reduced in B cell deficient mice upon influenza and MVA infection, but not upon VACV infection. Direct IFNAR signalling of B cells was necessary to trigger proper T cell activation and MHC-I upregulation, thus licensing B cells to promote CD8+ T cell expansion.

RESULTS

Patients suffering from rheumatic diseases are frequently treated with rituximab. Rituximab has a high depletion efficiency, which lasts for approximately 6 months (figure 1A). During a therapy cycle, vaccination against seasonal influenza is recommended, whereas the protective efficacy of influenza vaccination under conditions of B cell depletion is debated. To study the impact of B cell depletion on the induction of CD8+ T cell responses, rituximab-treated RA patients and healthy controls were human leucocyte antigen (HLA)-typed and vaccinated with Influvac. Influenza-specific T cells were determined 7 days post vaccination (figure 1B online supplemental figure 1). An increase of influenza-specific CD8+ T cells was observed in healthy individuals, but not in B cell deficient patients (figure 1C). To directly compare T cell responses of different donors, the fold induction of specific T cells post vaccination was calculated (figure 1D). Of note, the observed reduced T cell expansion in rituximab-treated patients was independent on other immunomodulatory colomedication (online supplemental figure 2). Thus, B cell depleted RA patients show reduced CD8+ T cell expansion upon anti-influenza vaccination. During the current SARS-CoV-2 pandemic, such patients are particularly vulnerable and bare an enhanced mortality risk. COVID-19 vaccination of younger patients just started and is applied independently of the rituximab treatment cycle, as similarly done for influenza vaccination. One patient with granulomatosis and polyangiitis (GPA) was analysed 4 weeks after second BNT162b2 vaccination for anti-SARS-CoV-2 antibody titres (figure 1E). In contrast to healthy controls, who mount high anti-SARS-CoV-2 IgG responses, no antibody titre was detected in the serum of this patient. Of note, as SARS-CoV-2 specific HLA-multimers are not available yet, T cell expansion could not be tested.

Since the analysis of immune responses in RA patients is potentially confounded by generally impaired immune status due to primary diseases and concomitant immunomodulatory treatment, the molecular mechanism of how B cells affect CD8+ T cell expansion was further addressed in B cell-deficient mice. To this end, JHT mice, in which the deletion of the J elements of the immunoglobulin heavy chain locus (JHT) resulted in a premature block of B cell development, were analysed. Upon infection with the mouse adapted influenza strain PR8, JHT mice showed significantly reduced expansion of nucleoprotein- and polymerase acidic protein-specific CD8+ T cells when compared with wild type mice (figure 2A,B). Thus, B cells are needed to efficiently induce influenza-specific CD8+ T cell responses in humans and mice.

To analyse whether the impact of B cells on T cell expansion is a unique feature on influenza infection, wild-type mice and JHT mice were infected with VACV, which is known to induce particularly strong T cell responses. The expansion of VACV-specific T cells was measured using an major histocompatibility complex (MHC)-I multimer loaded with the immune-dominant peptide B8. Upon VACV infection, wild type and JHT mice showed similar T cell expansion (figure 2C). Following MVA infection the expansion of B8-specific CD8+ T cells was significantly increased in wild type mice compared with JHT mice (figure 2D). To analyse whether B cell reconstitution of B cell-deficient mice restored T cell responses, splenic B cells of wild type mice were adoptively transferred into JHT mice 1 day prior to MVA infection. In B cell-reconstituted JHT mice the expansion of B8-specific CD8+ T cells was comparable with that in wild type mice (figure 2E), whereas adoptive transfer of serum from wild type mice, which contains natural antibodies but no B cells, had no impact (figure 2F). These data indicate that B cells support the induction of B8-specific CD8+ T cell responses on MVA infection, whereas during VACV infection B cells are not needed. Thus, the capacity of B cells to modulate CD8+ T cell responses is dependent on the properties of the pathogen/vaccine.

MVA and VACV induce distinct cytokine milieu upon infection: While MVA induces systemic IFN-I responses, VACV efficiently inhibits systemic IFN-I responses and rather induces an IL-12 dominated cytokine milieu. To test whether IFN-I responses affect B and T cell responses, we made use of conditional CD19-Cre IFNARflox/flox mice (IFNAR-B) in which the IFNAR is selectively deleted on B cells. Upon VACV infection, the expansion of B8-specific CD8+ T cells was similar in IFNAR-B and wild type mice (figure 3A), whereas upon MVA infection the expansion of T cells was significantly reduced in IFNAR-B mice (figure 3B). To test whether B cells are directly triggered by IFN-I, B cells from Mx2-luc reporter mice expressing a luciferase reporter upon IFNAR triggering were adoptively transferred into albino C57BL/6 mice. Upon MVA infection, a strong luciferase signal was detected by in vivo imaging particularly in the spleen and lymph nodes, which declined within the following day (figure 4). These results indicated that B cells were directly
triggers by IFN-I early after MVA infection, which is in accordance with the fast onset of MVA-induced IFN-I responses.21

To study effects of direct IFNAR signalling, B cells were isolated from spleens of MVA-infected wild type and IFNAR-B mice and analysed for differential gene expression by RNA sequencing. B cells of wild type mice expressed higher messenger RNA (mRNA) levels of MHC-I, β-2-microglobulin, and Ly6C than B cells of IFNAR-B mice (figure 5A). Furthermore, IFNAR-deficient B cells highly upregulated many chemokine receptors as well as CXCL1, CXCL9, and CXCL13, while CXCL10 was down-modulated when compared with wild type B cells (figure 5B). Thus, direct IFNAR-triggering of B cells modulates pathways involved in antigen presentation and tissue homeostasis.

To test whether virus-specific CD8+ T cells showed distinct chemokine receptor expression, MVA-specific T cells were sorted by fluorescence activated cell sorting (FACS) using an MHC-I multimer and mRNA was sequenced. Of note, no differences in chemokine receptor expression were found comparing B8-specific CD8+ T cells of wild type and IFNAR-B mice (figure 5C). Even being less frequent, B8-specific CD8+ T cells showed very similar gene expression profiles when compared with T cells from wild type mice.

In accordance with sequencing data, B cells’ surface expression of MHC-I and the B8 presenting haplotype H2-Kb was significantly increased upon direct IFNAR triggering, while MHC-II expression was upregulated upon infection IFNAR-independently (figure 6A–C). In addition, MVA infection induced CD86 and CD69 expression on wild type B cells, which was significantly reduced on IFNAR-deficient B cells (figure 6D–E). Thus, direct IFNAR signalling activates B cells and induces the expression of MHC-I as well as costimulatory molecules, and thus has a major impact on the capacity for antigen presentation of B cells.

DISCUSSION

Here, we report that B cell depletion can affect the expansion of virus-specific CD8+ T cells, depending on the T cell stimulating
**Rheumatoid arthritis**

The underlying mechanism is mediated via direct IFNAR signalling of B cells, which showed enhanced MHC-I, CD69, and CD86 expression, increased activation, and a distinct chemokine expression profile.

Most RA patients treated with rituximab received an immunomodulatory comedication and thus are therapeutically immunosuppressed. Since rituximab is not licensed as first-line RA treatment, the patients received other immunomodulatory treatments earlier. Additionally, RA patients were recently shown to harbour exhausted CD4+ T cells, which might influence the outcome of CD8+ T cell responses as well. Furthermore, patients are not immunologically naïve, since they were previously vaccinated against seasonal influenza virus or were in contact with the pathogen itself. The analysis of T cell expansion upon vaccination reflects a reactivation of memory CD8+ T cells rather than a primary response. The question remains, whether upon other diseases than RA B cell depletion influence CD8+ T cells responses as well. To prove that reduced expansion of CD8+ T cells in patients treated with rituximab was not caused by such secondary effects, we studied the result of B cell depletion on T cell responses in mice.

Here, we report a reduced in vivo expansion of antigen-specific CD8+ T cells in B cell-deficient mice upon infection with different viruses, suggesting the presence of a species-independent

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**Figure 2** B cell deficient mice show reduced virus-specific CD8+ T cell response upon influenza and MVA, but not VACV infection. (A) Wild type (WT) and JHT mice were infected with 5×10^5 ffu mouse adapted influenza virus for 7 days. (B) Influenza-specific CD8+ T cells were determined by using nucleoprotein (NP) or polymerase acidic protein (PAP) specific MHC-I multimers. WT and JHT mice were infected with 10^3 pfu of (C) VACV or (D) MVA and B8-specific CD8+ T cells were determined by using a MHC-I multimer. Data shown are pooled from 2 to 3 experiments with n=3–4. JHT mice were reconstituted with (E) 10^7 B cells or (F) 300 µL serum of WT mice 1 day prior to MVA infection and B8-specific T cell expansion was monitored. One out of two independent experiments is shown. Error bars indicate mean±SD; *p≤0.05, ***p≤0.001; one-tailed Mann-Whitney U test. MVA, modified vaccinia virus Ankara; ns, not significant; VACV, vaccinia virus.
mechanism of immune cell cross-talk. This phenomenon is remarkable, as dendritic cells (DCs) are broadly accepted to be the main APC responsible for T cell priming.

Guo et al showed that on anti-CD20 treatment, splenic CD8+ T cell proliferation was inhibited in a murine model of ITP.10 In that study, B cell depletion led to increased numbers of FOXP3+, CD4+, and CD8+ T cells within the spleen and lymph nodes, while splenic CD8+ T cells showed a reduced proliferation upon in vitro stimulation.10 In our experiments, the impaired T cell expansion was restored by adoptive transfer of B cells. B1 cell-derived natural antibodies, which are present in the serum of naïve mice, were shown to decorate antigen rather unspecifically and to enhance antigen presentation by antigen trapping.23 However, we found that serum transfer was not effective in restoring the deficit in CD8+ T cell expansion in B cell deficient mice.

Upon MVA infection, the lack of IFNAR expression exclusively on B cells resulted in reduced T cell expansion as similarly detected in B cell deficient mice. Thus, besides serving as a direct third signal for T cell responses15 IFN-I can also increase CD8+ T cell responses indirectly via B cells. IFN-I responses were shown to critically modulate the overall cytokine milieu and in particular, to inhibit IL-12 responses.15 24 25 Furthermore, IL-12 was shown to serve as third signal in T cell activation as well,26 27 which might explain why in the absence of IFN-I responses B cells are dispensable for CD8+ T cell expansion.

Direct IFNAR triggering on B cells induced the activation of the STAT1 pathway and enhanced the expression of Ly6C and CD69. Moreover, MHC-I and CD86 were induced, thus facilitating adequate antigen presentation. Interestingly, B cells were described before to cross-present MHC-I restricted antigen, although less efficiently than DC.30 Thus, IFN-I is a key mediator to promote efficient interaction between B cells and CD8+ T cells. Of note, virus-induced IFN-I was also reported to confer disintegration of B cell follicles30 and to drive B cell reduction by differentiating B cells into short-lived antibody-secreting cells.30 This mechanism called ‘B cell decimation’ was independent of B cell-intrinsic IFN-I sensing.30

**Figure 3** IFNAR depletion on B cells affects B8-specific CD8+ T cell responses upon MVA, but not VACV infection. Wild type (WT) and CD19-Cre+IFNAR−/− (IFNAR-B) mice were infected with 10^5 pfu (A) VACV or (B) MVA. B8-specific CD8+ T cells were determined by using an MHC-I multimer. Data shown are pooled from 3 to 4 experiments with n=3–4. Error bars indicate mean±SD; ***p≤0.001; one-tailed Mann-Whitney U test. IFNAR, type I interferon receptor; MVA, modified vaccinia virus Ankara; ns, not significant; VACV, vaccinia virus.

**Figure 4** MVA-induced IFN-I responses directly trigger B cells in vivo. 10^5 B cells isolated from Mx2-luc reporter mice were adoptively transferred into albino C57BL/6 wild type mice 1 day prior to infection. Upon treatment with phosphate buffered saline (PBS) (first mouse per row) or infection with 10^5 pfu MVA (mouse 2–4 per row), luciferase reporter expression in adoptively transferred B cells was monitored after luciferin administration by in vivo imaging at different days (d) post infection (scale=p/sec/cm^2/sr). one out of two independent experiments is shown. IFN-I, type I interferon; IFNAR, IFN-I receptor; MVA, modified vaccinia virus Ankara.

**Figure 5** MVA-induced IFN-I responses activate B cells, but do not affect CXCR5+ CD8+ T cell responses. Wild type (WT) and IFNAR-B mice were infected with 10^5 pfu MVA and B cells were isolated 1 day post infection via untouched magnetic cell separation and prepared for mRNA sequencing. Differentially regulated (A) surface molecules and (B) chemokine as well as chemokine receptor expression profiles are shown. n=3 (C) WT and IFNAR-B mice were infected with 10^5 pfu MVA and B8-specific CD8+ T cells were FACs-sorted six days post infection from spleens using a B8-specific MHC-I multimer. RNA sequencing samples were pooled from three different mice and chemokine expression profiles were analysed. IFN-I, type I interferon; IFNAR, IFN-I receptor; MVA, modified vaccinia virus Ankara.
Figure 6  MVA-induced IFN-I responses modulate antigen presentation in B cells. Wild type (WT) and IFNAR-B mice were infected with 10^5 pfu MVA and splenocytes were isolated 48 hours post infection. Expression of (A) MHC-II, (B) MHC-I, (C) H2-ka, (D) CD86, and (E) CD69 was analysed by flow-cytometry. Data shown are pooled from 2 to 3 experiments with n=2–4. Error bars indicate mean±SD; *p≤0.01; ***p<0.001; one-tailed Mann-Whitney U test. DPI, days post infection; IFN-I, type I interferon; IFNAR, IFN-I receptor; MVA, modified vaccinia virus Ankara; MFI, mean fluorescence intensity; NS, not significant.

Whether B cells and CD8+ T cells are in direct contact within secondary lymphoid organs has been discussed controversially. B cell follicles and T cell zones are organised in separate compartments in secondary lymphoid organs. In human (HIV) and simian immunodeficiency virus (SIV) infection, B cell follicle sanctuaries were shown to permit a persistent infection reservoir due to the absence of protective CD8+ T cell responses. Of note, rituximab treatment of RA patients not only depletes B cells, but also CD20+ terminal differentiated T cell subset with immune-regulatory and proinflammatory function. Nevertheless, the frequency of CD20+CD8+ T cells is very low in humans and might not be the primary cause for reduced T cell expansion in rituximab-treated patients.

Here, we studied the immune response against an influenza vaccine in B cell depleted RA patients. It is possible that antigen-specific T cell responses are also reduced in rituximab-treated patients after vaccination against other diseases. Of note, SARS-CoV-2 infection induces only mild IFN-I responses due to active IFN-I blockade and patients with severe COVID-19 displayed a highly impaired IFN-I response when compared with patients with moderate COVID-19 courses. Among the available COVID-19 vaccines, the mRNA-based vaccines induce IFN-I dominated cytokine milieus. In contrast, for adenovirus-based vaccines it was shown that excessive IFN-I responses rather inhibit transgene expression, and as a consequence, vectors inducing only minor IFN-I responses were chosen for the development of an immunogenic vaccine. Among SARS-CoV-2 adenoviral vectors, HAd5-based vaccines most likely induce less IFN-I compared with ChAdOx1-based vaccines. Considering a reduced CD8+ T cell response in the presence of IFN-I with simultaneous absence of B cells, the non-IFN-I inducing adenovirus based vaccines could be even better suited to induce decent CD8+ T cell responses in B cell-depleted patients compared with mRNA-based vaccines.

Patients treated with rituximab were reported to bare an enhanced mortality risk if infected with SARS-CoV-2. With regard to COVID-19 disease, it appears therefore not advisable to delay vaccination of such patients a few months after rituximab suspension, when naïve B cells have repopulated. In contrast to other vaccines, COVID-19 vaccine should rather be administered as soon as available. In order to induce at least protective CD8+ T cell responses, the usage of vaccines inducing a cytokine milieu that is not dominated by IFN-I could be beneficial for such patients.

MATERIAL AND METHODS

Patients and healthy controls

After immunisation with Influenza season 2012/2013 or 2013/2014 (Mylan Healthcare) PBMC were isolated on day 0 and 7, and frozen at −80°C. The frequency of influenza virus specific CD8+ T cells was determined using HLA matched pentamers (Proimmune) (online supplemental table 1). Five RA patients (one male, four female, average age 63 years) and 10 healthy controls (five male, five female, average age 31 years) were identified with one or more matching HLA subtypes. After BNT162b2 vaccination, 1 GPA patient (female, age 20 years) and four healthy controls (two female, 1 male, average age 33 years) were recruited. Characteristics of patients are indicated (online supplemental table 2).

Mice

C57BL/6 (wild type) and albino C57BL/6BrdCrHsd-Tyrc (C57BL/6 albino) mice were purchased from Harlan Winkelmann or Envigo. IFNARfl/flJHT, 5D19-CreIIFNARfflox/fflox (IFNAR-B), and Mx2-luc reporter mice were described before. All mice were bred under specific pathogen free conditions at the central animal facility of TWINCORE and the Helmholtz Centre for Infection Research, Brunswick, Germany, or the Paul-Ehrlich-Institut, Langen, Germany. Mouse experimental work was carried out using 8 to 16 week old mice in compliance with regulations of the German animal welfare law (F107/64, 09/1655, 10/0265, 10/0266, 11/0367, 12/0939, 13/1073).
Viruses and infection
MVA and VACV strain Western Reserve (originally provided by Bernard Moss, NIH, Bethesda, Maryland, USA) were propagated and titrated on chicken embryonic fibroblasts and purified by sucrose density gradient centrifugation. Mouse-adapted influenza A/PR/8/34 (H1N1 PR8) was propagated in the chorioallantoic fluid of 10 days old pathogen free embryonated chicken eggs at 37°C and was kindly provided by Dr. P. Blazejewska, Dr. K. Schughart, and Carlos A. Guzmán (Helmholtz Centre for Infection Research, Brunswick, Germany). In all infection experiments, mice were treated with 10^5 pfu MVA/VACV, or 5×10^5 pfu influenza virus dissolved in PBS intravenously.

Adaptive cell and serum transfer experiments
B cells were isolated from spleens, via untouched B cell separation kit (Miltenyi). 10^5 B cells with a purity of 90%–98% were adoptively transferred into recipient mice. For serum transfer, 300 μl serum pooled from different wild-type animals was injected 1 day prior to infection.

In vivo imaging
Reporter mice were intravenously injected with 3 mg of D-luciferin (PerkinElmer) diluted in PBS and anaesthetised using 2.5% isoflurane (Abbott). The emitted light signals were measured in the in vivo imaging system IVIS SpectrumCT (Calliper) and analysed with Living Image 4.5 software (Calliper).

Flow cytometric analysis and cell sorting
All antibodies were purchased from eBioscience or BD-Pharmergen. Cells were measured using flow cytometry (LSR II, BD) and data were analysed by FlowJo software. FACS sorting was conducted using a MoFlo XDP cell sorter (Becton Dickinson).

ELISA
Anti-SARS-CoV-2 IgG antibody titres were determined from serum using an ELISA (Euroimmun AG, EI 2606–9601 G) according to the manufacturer’s instructions. The ratio of the optical density to the calibrator was used to classify the samples as negative (ratio <0.8) or positive (ratio ≥1.1).

Deep sequencing and pathway analysis
After 24 hours of MVA infection, B cells were isolated from spleens of C57BL/6 and IFNAR-/- mice using the untouched magnetic B cell separation kit (Miltenyi). FACS sorting of B8-specific CD8^- T cells from spleens was conducted using a MoFlo XDP cell sorter (Becton Dickinson). After RNA isolation using tNucleaseSpin RNA kit (Macherey-Nagel) mRNA sequencing was performed at TRON (Translational Oncology Mainz, Germany). Pathway analysis was performed as described in online supplemental methods section.

Statistical analysis
Statistical analyses were performed using GraphPad Prism V6 software as indicated.

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Competing interests
None declared.

Patient consent for publication
Not required.

Ethics approval
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All data relevant to the study are included in the article or uploaded as supplementary information. All data relevant to the study are included in the article or uploaded as supplementary information.

Supplemental material
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