

Multifocal lymphadenopathies with polyclonal reactions primed after EBV infection in a mRNA-1273 vaccine recipient

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Summary

We report a case of recurrent tender, multifocal lymphadenopathies associated with B-symptoms, clinically mimicking lymphoma in a mRNA-1273 vaccine recipient after a recent Epstein-Barr virus (EBV) infection.

In the lymph node biopsy, monocytoid B-cell hyperplasia, TH2 (GATA3+) predominance, and hyperplasia of interferon-gamma-producing plasmacytoid dendritic cells were observed along with sustained neutralising antibody production against SARS-CoV-2 wild-type and five variants. High titres of anti-S antibodies and neutralising antibodies were observed, excepted for variant B.1.529** (omicron) and B.1.351** (beta), due to several mutations in the spike protein, including the E484K mutation.

We postulated that EBV acted as an immunological enhancer with the mRNA-1273 vaccine, inducing a sustained inflammatory response over several weeks. However, the polyclonal nature of the lymphadenopathy with polytypic plasmacytosis and pseudo-tumoural reaction cell hyperplasia were associated with failure to mount acute phase responses.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing coronavirus disease 2019 (COVID-19) with a wide range of polymorphic clinical manifestations and outcomes. Messenger RNA (mRNA)-based vaccines against SARS-CoV-2 have an overall excellent safety profile, but can be associated with unusual adverse reactions, such as diffuse adenopathy that may be mistaken for malignancy [1]. Most adverse reactions following immunisation are mild, local and self-limiting. Systemic adverse reactions include fatigue, lymphadenopathies, fever, myalgia, appendicitis, myocarditis, Guillain-Barré syndrome or Bell's palsy. Cases of herpes simplex and zoster have been reported after mRNA COVID-19 vaccines, but no evi-

dence of oropharyngeal shedding of herpesviruses was observed within one week after immunisation [2, 3].

Case description

A 44-year-old woman, indoor cat owner (5-year-old), suffered in mid-February 2021 from acute fatigue, palpable spleen and tonsillitis with white spots, diagnosed as mononucleosis based on Epstein-Barr virus (EBV) serology. She took no medication, had no relevant medical history, and had tested negative for EBV with a previous hospitalisation for pneumonia and dysaesthesia with spontaneous resolution.

Early in March 2021, the patient received her first mRNA-1273 SARS-CoV-2 vaccine (left deltoid) and after 48–72 hours, she presented with new left-sided supraclavicular lymphadenopathy, fever, night sweats and weight loss (–4% of total body weight in 15 days).

On examination, the patient presented with fever (up to 39 °C), multiple tender cervical, supraclavicular and axillary lymph nodes. Initial investigations found a white blood cell count of $7.1 \times 10^9/l$ with reactive lymphocytes and stable C-reactive protein levels between 6.0 and 7.9 mg/l (reference range 0–8 mg/l). Routine biochemistry including kidney and liver function tests were within normal limits.

Ultrasonography revealed large supraclavicular lymph nodes, one $17 \times 13 \times 10$ mm (fig. 1A). A contrast-enhanced computed tomography (CT) scan confirmed enlarged axillar and supraclavicular lymphadenopathies. Smear from a fine needle aspiration revealed a florid reactive lymphadenopathy pattern with tingible body macrophages and lymphocytes at different stages of maturation.

One month after the first vaccination, the second mRNA-1273 vaccine dose was administered, followed within 24 hours by a more pronounced reaction with extension to contralateral lymph nodes associated with B-symptoms that resolved after 6 weeks. Given the progression of

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symptoms and to rule out a malignant lymphoproliferative disease, excisional biopsies of the supraclavicular and cervical nodes were performed. Histopathology showed follicular and monocytoid B-cell hyperplasia, a TH2 (GATA3+) predominance with hyperplasia of interferon-gamma-producing plasmacytoid dendritic cells (fig. 1B-D), and a moderate polyclonal plasmacytosis (figure 2).

The clinical picture was consistent with an acute inflammatory state and reflects abnormal reaction in the background of a primed adaptive immune system. Immunohistochemical staining for SARS-CoV-2 N-antigen, adenovirus, cytomegalovirus (CMV), herpes simplex virus 1 and 2, human herpes virus 8, parvovirus B-19, varicella zoster virus, toxoplasma- and spirochaete-antigens were negative. *In situ* hybridisation for EBV small RNA (EBER) showed scattered (maximum 2%) positive parafollicular immunoblastoid cells.

Bartonella henselae, *Toxoplasma gondii*, CMV, adenovirus IgG and IgM were all negative. There was no serological evidence of previous SARS-CoV-2 infection (absence of anti-nucleocapsid antibodies), but high-titre neutralising antibodies and anti-spike antibodies were detected (Luminex® assay), as expected after immunisation. The patient developed sustained SARS-CoV-2 antibody production for wild type and five main variants: 18 and 57 days

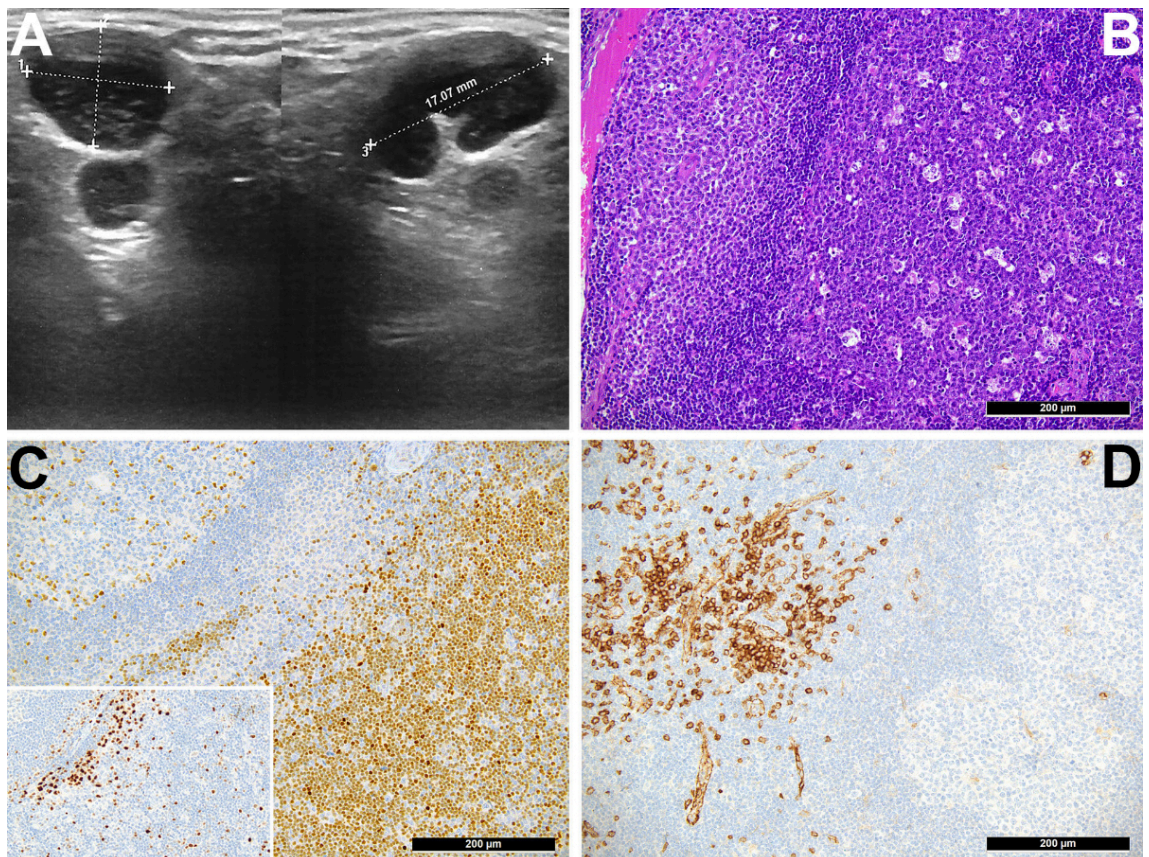
after the first vaccination, the neutralising activities were 9-fold and >11-fold higher than the threshold. Eight months after the first vaccination, the neutralising activities against wild type SARS-CoV-2 and the main variants decreased significantly, but they still remained above the neutralising threshold, except for the variant B.1.131** (table 1).

In mid-December 2021, reckoning the increasing need for protection from more contagious SARS-CoV-2 variants, the patient decided to receive a booster vaccine, but with only the half dose of the standard mRNA BNT162b2. Despite a previous positive rechallenge (second immunisation) after the acute EBV infection, she had neither clinical signs, nor reported symptoms, excepted a mild local reaction 12 hours following the injection.

Discussion

The patient presented with severe relapsing systemic post-vaccination lymphoma-mimicking reactions lasting for several weeks and leading to invasive diagnostic procedures. In a nationwide cohort study, BNT162b2 mRNA COVID-19 vaccine was associated with an increased risk of lymphadenopathy (risk ratio 2.43, 95% confidence interval [CI] 2.05–2.78; risk difference 78.4 events per 100,000 individuals, 95% CI 64.1–89.3), as well as my-

Figure 1: A. Ultrasonography revealed large supraclavicular lymph nodes of 17 × 13 × 10 mm. B. Medium power view of the enlarged lymph node showing follicular hyperplasia (dark purple cells intermingled with starry sky-appearing tingible body macrophages on right part) and monocytoid/plasmacytoid parafollicular hyperplasia (paler cell collection on the left side); Haematoxylin eosin stain, original magnification 200×. C. Significant T-helper 2 cell-skewing in the paracortex shown by the positive staining of the respective cellular compartment for GATA3, compared with the lower presence of T-helper 1-equivalents in the same area in the staining for T-bet/TBX21 (insert); immunoperoxidase staining, original magnification 200×. D. Significant increase of CD123-positive interferon-gamma-producing plasmacytoid dendritic cells in the parafollicular compartment; immunoperoxidase staining, original magnification 200×.



ocarditis (risk ratio 3.24) and appendicitis (risk ratio 1.40) [2]. Generally, lymphadenopathies are considered as an indicator of successful immunisation as they are intrinsically linked to vaccine-induced activation of the immune system [4]. In the present case, the patient developed sustained antibody production against SARS-CoV-2 wild type and variants. The recent EBV infection likely contributed to these reactions, serving as a primer: systemic reactions associated with EBV, polytypic B-cell proliferation and lymphoid hyperplasias are reported in several situations, such as in the context of injuries, sun exposure, exposure to certain drugs, mosquito bites and other vaccinations, which may all serve as triggers [5]. Together with fever, lymphadenopathy is a well-known adverse reaction following

immunisation with various vaccines, including measles-mumps-rubella, human papilloma virus, influenza, hepatitis B virus, smallpox or Calmette-Guérin bacilli [5–7]. EBV is a carcinogenic virus, associated with various lymphomas, as well as immunostimulation [8]. EBV nuclear antigen 1 (EBNA1) is able to recruit pro-inflammatory and immune-stimulating macrophages, histiocytes and TH2 cells, which secrete a wide range of interferons, interleukins and other cytokines that contribute to the development of lymphadenopathies with B-symptoms. Unfortunately, EDTA blood samples were not available from the time of the major post-vaccination syndrome, which precluded our study of the EBV-DNA load dynamics during this post-vaccination illness. Further, since a small proportion of

Figure 2: Polyclonal plasmacytosis (kappa brown; lambda red).

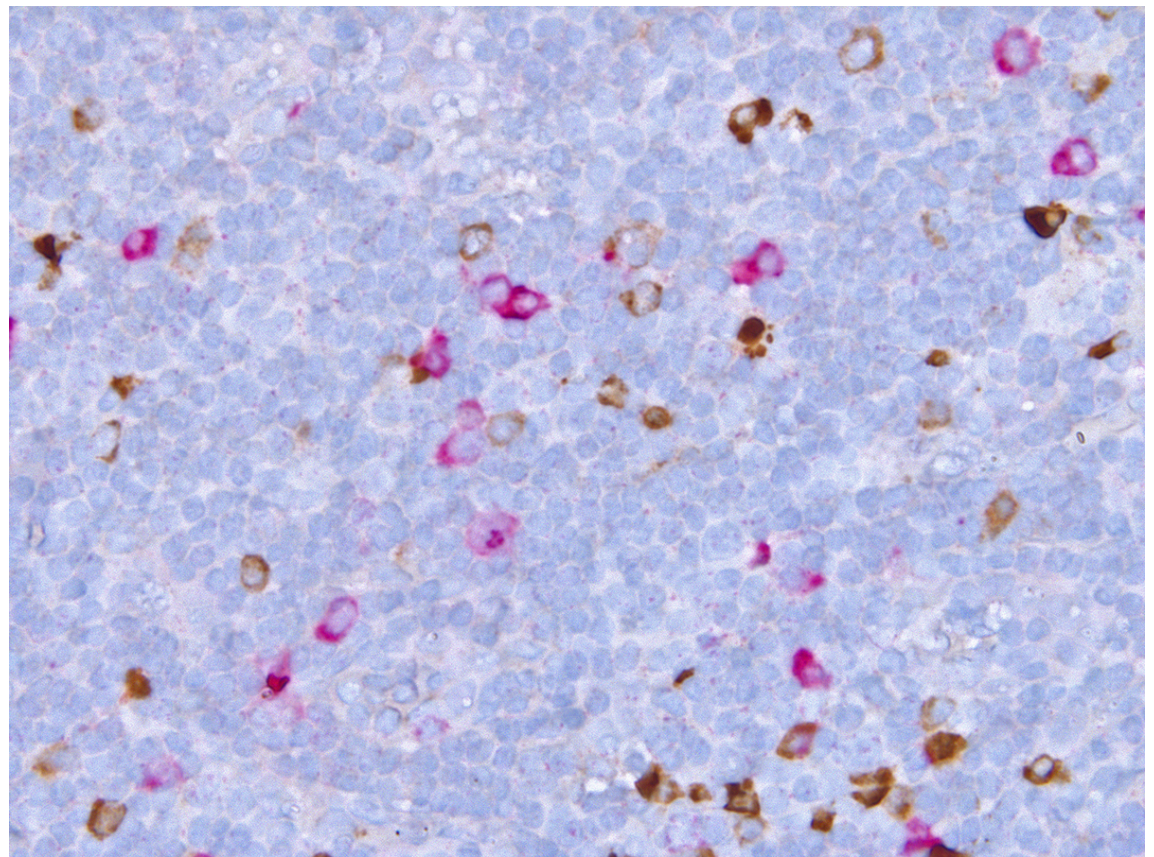


Table 1:

IgG antibodies directed against the spike protein (S) or the nucleocapsid protein (N) of the SARS-CoV-2 virus, assessed using a Luminex[®] assay (10) and neutralising antibodies towards the spike protein of the wild type virus and of various SARS-CoV-2 variants. Note the expected high titres of anti-S antibodies and heterogeneously high titres of neutralising antibodies after SARS-CoV-2 vaccination. These neutralising antibodies appear, however, to exhibit reduced efficacy on variant B.1.529** (omicron) and B.1.351** (beta), as expected owing to several mutations in the spike protein, including the E484K mutation.

Day after vaccination	IgG antibodies against the SARS-CoV-2 virus		Neutralising antibodies against the spike protein of the wild type SARS-CoV-2 wild type (WT) and five variants					
	Spike	Nucleocapsid	WT	B.1.1.7** (alpha)	B.1.351** (beta)	P.1** (gamma)	B.1.617.2** (delta)	B.1.529** (omicron)
D0 (March 2021)	First vaccine (standard dose)							
D18	113	NEG*	448	303	46	159	99	39
D28	Second vaccine (standard dose)							
D57	115	NEG*	564	397	121	280	200	73
D240	55.1	NEG*	103	79	44	67	57	–
D290	Third vaccine (half dose)							
D365 (March 2022)	81.7	NEG*	1806	1136	552	1269	833	513

* NEG: negative Luminex anti-SARS-CoV-2 serology; please note that with our assay, the neutralising activity is considered as absent below 50, low between 50 and 100, moderate between 100 and 150, and strong above 150.

adults (1–5%) fail to mount anti-EBNA IgG, we cannot formally exclude the possibility that the present EBV serology represents a reactivation or presence of persisting irrelevant cross-reactive IgM. At least, the anti-EBV IgM were not cross-reactive in the setting of a syphilis or human immunodeficiency virus (HIV) primary infection. *Bartonella henselae* and *Toxoplasma* were included in the differential diagnosis since both may be acquired by exposure to cats and both cause lymphadenopathy, but the negative serology and the negative immunohistochemistry excluded these two hypothetical causative agents.

The histopathological appearance of the patient's lymphadenectomy specimen indicated a predominance of TH2, polyclonal plasmacytosis, hyperplasia of B cells, and plasmacytoid dendritic cells that is consistent with the clinical picture of a protracted evolution of an abnormal immune response over several weeks.

We postulate that recent EBV infections primed the lymph node microenvironment of lymphocytes and macrophages, providing high levels of cytokines and/or growth factors, and decisively contributed to enhanced immune-stimulation after application of the mRNA-1273 vaccine. As for EBV, the conjunction of recent infections with other lymphotropic viruses (e.g., CMV, HHV6, SARS-CoV-2), medications (e.g., drug rash with eosinophilia and systemic symptoms-DRESS syndrome) or mRNA-1273 SARS-CoV-2 vaccination are likely to predispose to major lymphoma-mimicking reactions [9]. Thus, since there was no systemic reaction after the third immunisation with another mRNA COVID-19 vaccine 9 months later, EBV involvement as primer seems likely in the first and second systemic reactions following vaccination. Failure to mount acute phase responses (e.g., relatively low CRP level) is not uncommon, even though there is a rise of multifocal lymphadenopathies associated with B-symptoms. This discrepancy might be explained by the polyclonal nature of the lymphadenopathy and pseudo-tumoural B-cell reaction with interferon-gamma production.

In our patient, adenopathy completely resolved with no sign of malignancy. One year after vaccination, IgG and neutralising antibodies against the spike protein were still above the half-maximum effective concentration threshold (EC50) for neutralising activity of SARS-CoV-2 wild type and four variants (variant alpha B.1.1.7**; variant gamma P.1**; variant delta B.1.617.2**; variant omicron B.1.529**) [10].

The causal relationship between the mRNA COVID-19 vaccine and the development of lymphadenopathies with B-symptoms was estimated as certain, considering the mechanistic and temporal relationship plausibility, as well as the positive rechallenge. The absence of systemic reaction after the third immunisation (ie, 10 months after the primary infection) strengthens the role of viral factors as causative agent. In conclusion, EBV could act as an immunological primer, sustaining a cascade of inflammatory processes with polytypic B-cell proliferations and hyperplasia of interferon-gamma-producing plasmacytoid dendritic cells.

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Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest was disclosed.

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