



Case Report

# Transient Induction of HLA-DR Expression in Polymorphonuclear Neutrophils Following the mRNA Vaccine BNT162b2 (Comirnaty): A Case Report

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#Equally contributed to the study.

## Case Report

A flow cytometry assay, to be used as an improved diagnostic test to evaluate infections, is currently under scrutiny at our institution. This assay relies on monitoring changes that occur in the expression of CD64 (Fcγ-receptor-1) on circulating polymorphonuclear neutrophils (PMN), and of CD169 (sialoadhesin or siglec-1) and HLA-DR in circulating monocytes (MO). The assay uses fluorochrome-conjugated monoclonal antibodies to stain PMN and MO; modifications in fluorescence signal intensity are taken as measure of marker modulation [1]. The assay includes constructing a reference data set using anonymized samples from healthy subjects. Some subjects are also assessed longitudinally for consistency. During flow data analysis of a blood sample, we observed an unexpected marker expression profile, not consistent with that of healthy individuals. The sample belonged to a 49-year-old woman working as Healthcare Professional at the Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome (Italy) who had received her second dose vaccination with the messenger RNA vaccine BNT162b2 (Comirnaty) five days before sample collection. After the shot, she had experienced rapidly developing induration, muscle soreness and pain at the vaccination side radiating to the lower arm. Systemic symptoms included fatigue, chest tightness, joint pain, especially legs and lower back, headache, chilling and high fever (~103°F- 39.5°C), which promptly responded to low dose painkillers such as ibuprofen and paracetamol. Local and systemic reactions resolved over the following 2 to 3 days with no additional treatment other than paracetamol. Personal history included only mild Hashimoto thyroiditis for which she was on levothyroxine, 75 mg/die. No family history of interest was reported. Of note, she did not experience any local and systemic symptom after the first dose, with exception of transient mild local pain and tenderness. Because the concomitance of the adverse reactions and alteration in the PMN and MO phenotypic profile suggested a causative effect of the vaccination, we reevaluated a blood sample that had been collected a few days before first vaccination for the

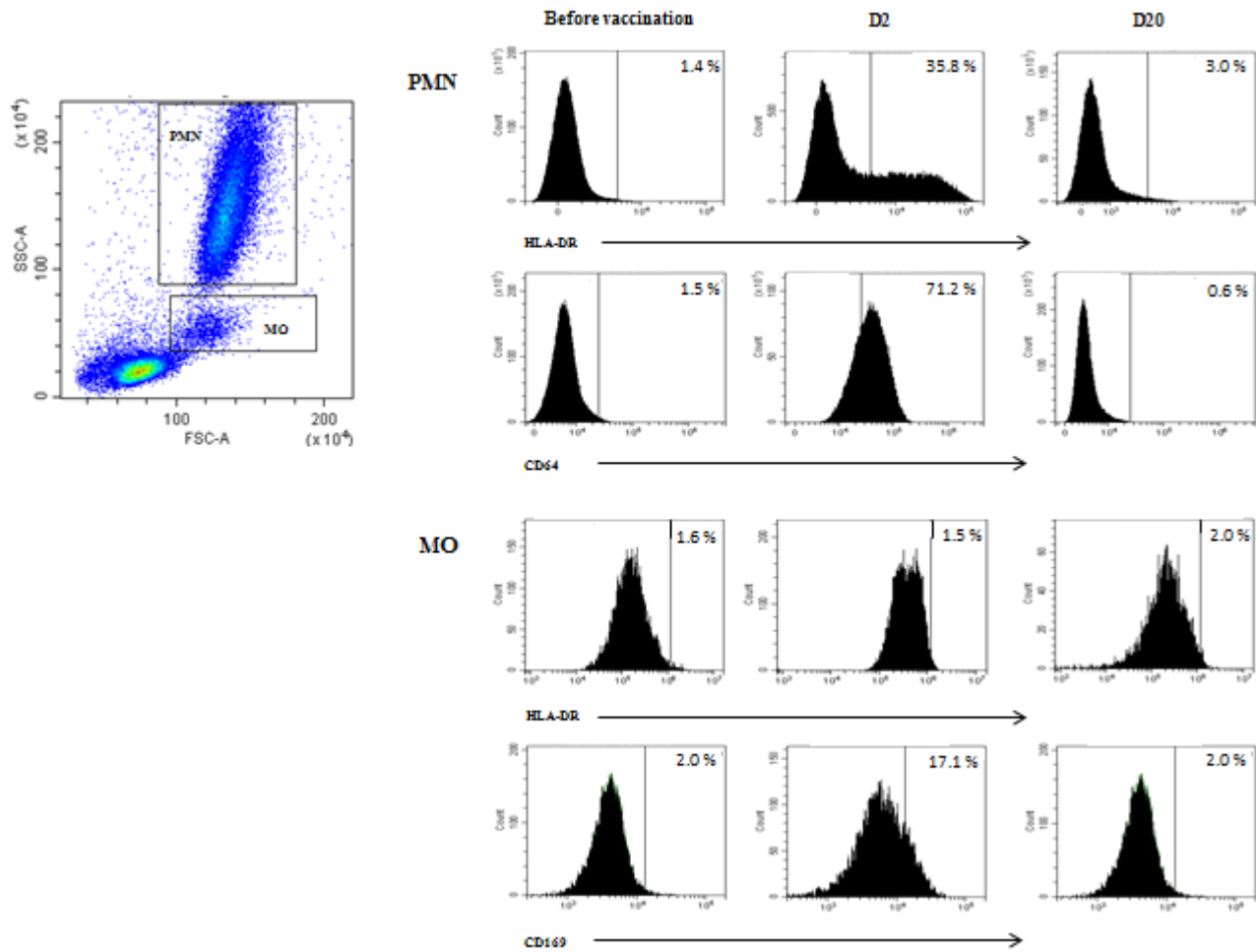
longitudinal evaluation of the flow cytometry test performance. We also collected an additional blood sample 20 days after the second shot. Flow cytometry profiles of PMN and MO in the three blood samples are shown in the Figure. PMN and MO population were selected on typical physical parameters, i.e., forward and side scatter (FSC and SSC, respectively) (Figure, upper left, pseudocolor dot plot). In the analysis of PMN, enhanced expression of HLA-DR and CD64 (upper and second from above histogram row, respectively) at day 2 after second vaccination compared with the pre-vaccination time was evident. The expression of both markers reverted to pre-vaccination value at day 20 after second vaccination. In the analysis of MO, no up-modulation of the constitutive HLA-DR expression on MO (lower histogram row) manifested, only a small proportion of dimly stained cells seemed to increase their fluorescence intensity following vaccination. Up-modulation of CD169 expression (third from the top histogram row) was evident. In analogy with PMN, all changes completely reverted to pre vaccination value at day 20. White blood cell differential was not modified by the vaccine (not shown).

The most unexpected finding was the appearance of HLA-DR on PMN surface following vaccination. These cells are key components of the innate immune system and act as short-lived phagocytes that clear invading bacterial and fungal pathogens with essentially no role in shaping adaptive immune response; consistently, PMN display no or very little HLA-DR at homeostasis [2]. However, *in vitro* studies have shown that PMN can be induced to express surface HLA-DR molecules, which are normally contained in cytoplasmic stores, when exposed to certain inflammatory cytokines, e.g., IFN-γ and GM-CSF [3]. These observations are consistent with the presence of HLA-DR expressing PMN in patients with rheumatoid arthritis [4] or Wegener's granulomatosis [5], two chronic inflammatory diseases associated with high levels of inflammatory cytokines.

Thus, it can be hypothesized that the HLA-DR expressing PMN we found here had been activated by the

inflammatory cytokines released upon vaccination. This view is supported by the observation that the increase in HLA-DR expression was paralleled by an increase in CD64 expression: this molecule is a typical indicator of activation upon

exposure to inflammatory cytokines [1] and experimental data have clearly indicated concomitant translocation of CD64 and HLA-DR molecules from cytoplasmic stores onto the surface of PMN following activation [6-7].



**Figure 1:** Samples are run in a CytoFLEX S flow cytometer and analyzed using the CytExpert software (Beckman Coulter). Shown is the flow cytometric analysis of results of the flow cytometry assay performed on blood samples collected at various time points, i.e., before first dose vaccination, and 2 (d2) and 20 (d20) days after second dose vaccination. Upper left corner, pseudocolor dot plot to identify PMN and MO by their characteristic position on the dot-plot of forward scatter (FSC, a measure of size) versus side scatter (SSC, a measure of granularity) and set gates around the two populations with visual help of the machine CytExpert software. Upper and second from above histogram row, expression of HLA-DR and CD64, respectively, within the PMN population. Third from the top histogram row and lower histogram row, expression of HLA-DR and CD169, respectively, within the MO population; The extent of modulation is represented as percentage of cells exceeding the threshold established before vaccination (vertical line in each histogram plot) and was computed using the CytExpert software.

The kinetics of CD169 modulation on MO showed a comparable behavior, being upmodulated at day 5 and returning to pre-vaccination value at day 20. Because CD169 appearance on MO surface indicates exposure to type I interferons (IFNs), especially IFN- $\gamma$  [1], we hypothesize that also MO were responding to vaccine-induced cytokines. Supporting this view, all changes in the surface marker expression were temporally related to the localized and the systemic adverse reactions ensuing vaccine administration, which are commonly regarded as the reflection of exuberant, transient production of inflammatory cytokines, especially type I IFNs [8]. Of note, CD169 expression by MO is currently held to be a biomarker to monitor disease progression and clinical outcome in COVID-19 patients as

this surface molecule has been reported as reliable indicator of antiviral molecule release, including IFN $\alpha$  [9].

The significance of the enhanced HLA-DR expression by PMN following SARS-Cov-2 vaccination remains to be clarified. It is possible that these activated PMN merely represent an epiphenomenon of the vaccine-induced cytokine release. However, it is tempting to speculate that the HLA-DR expressing activated PMN may in part mimic antigen presenting cell activity, as evidenced *in vitro* [10] and therefore contribute to vaccine response. In this scenario, it is worth remembering that production of type I IFNs, the likely culprits for BNT162b2 related adverse reactions, underlies both humoral and cytotoxic immune response production [8]. Intriguingly, serological assessment of the response to the

vaccine revealed the presence of anti-Sars-CoV-2 S-protein RBD [Atellica® IM SARS-CoV-2 IgG (sCOVG) assay] antibody level in the upper quartile (>150 index) among all healthy subjects vaccinated at our Institution, suggesting a relationship between the PMN and MO surface marker modulations and amplification of adaptive immune response.

Even though report of a single case may fall short in reflecting a generalized pattern, present observations show for the first time that a component of the innate immune system is involved in the response to the messenger RNA vaccine BNT162b2 (Comirnaty). These findings can possibly yield initial information for further studies on PMN impact to the SARS-Cov-2 vaccination and feed important information into future translational research.

## Declaration

The study received institutional ethical committee approval, conforming to the principles of the Declaration of Helsinki, and is registered as 331/2021. The subject gave informed consent.

## Conflict of Interest

The authors declare that they have no competing interests.

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