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# Lack of evidence for post-vaccine onset of autoimmune/lymphoproliferative disorders, during a nine-month follow-up in multiply vaccinated Italian military personnel

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ABSTRACT

Anecdotal case reports, amplified by mass media and internet-based opinion groups, have recently indicated vaccinations as possibly responsible for autoimmunity/lymphoproliferation development. Multiply vaccinated Italian military personnel (group 1, operating in Italy, group 2, operating in Lebanon) were followed-up for nine months to monitor possible post-vaccine autoimmunity/lymphoproliferation onset. No serious adverse event was noticed in both groups. Multivariate analysis of intergroup differences only showed a significant association between lymphocyte increase and tetanus/diphtheria vaccine administration. A significant post-vaccine decrease in autoantibody positivity was observed. Autoantibodies were also studied by microarray analysis of self-proteins in subjects exposed to ≥4 concurrent vaccinations, without observing significant difference among baseline and one and nine months post-vaccine. Moreover, HLA-A2 subjects have been analyzed for the possible CD8T-cell response to apoptotic self-epitopes, without observing significant difference between baseline and one month post-vaccine. Multiple vaccinations in young adults are safe and not associated to autoimmunity/lymphoproliferation onset during a nine-month-long follow-up.



*Abbreviations:* BCG, Bacillus Calmette-Guérin; CDC, Centers for Disease Control and Prevention; Td, tetanus/diphtheria; MMR, measles/mumps/rubella; HA, hepatitis A; HB, hepatitis B; T0, time 0; T1, time 1; T2, time 2; Ig, immunoglobulins; ANA, antinuclear antibodies; anti-dsDNA, anti-double-stranded DNA antibodies; anti-ENAs, anti-extractable nuclear antigens antibodies; APL, anti-phospholipid antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; RF, rheumatoid factor; HLA, Human leukocyte antigen; PCR-RDB, PCR and Reverse Dot Blot; commercial kit LIPA, -; PCR-SSP, DNA amplification using sequence specific primer; MFI, mean fluorescence intensity; IFN, interferon; IFI, indirect immunofluorescence; ELISA, enzyme-linked-immunosorbent assay; INGM, National Institute for Molecular Genetics; HSA, human serum albumin; Elispot, enzyme linked immunospot; ACTB, actin cytoplasmic 1; ROK, heterogeneous nuclear ribonucleoprotein; LAM1, lamin B1; MYH9, non-muscle myosin heavy chain 9; GDIS, rho GDP dissociation inhibitor 2; VIME, vimentin; PSA1, proteasome component C2; RLA, 60S acidic ribosomal protein P2; SFC, spot forming cells.

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## 1. Introduction

The armed forces of all countries in the world protect their personnel against the risk of infections by prophylactic vaccination programs in order to maintain operational readiness [1]. In fact, the military are particularly vulnerable to infectious diseases as a consequence of community life, often in precarious hygienic and hostile climatic conditions, and rapid relocations to areas with different epidemiology of infectious diseases for operational missions [2]. Despite the undeniable merits of vaccinations in the control of infectious diseases [3] and the substantial vaccine safety [4], recently, especially in developed countries, mass media have dedicated special attention to the potential vaccine-induced damages. The emergence of some cases of Hodgkin's and non-Hodgkin's lymphomas in otherwise healthy military personnel of the Italian armed forces has fueled the argument that vaccinations, specifically multiple vaccinations, may represent important co-factors for the induction of lymphoproliferative disorders. This climate of opinion has probably contributed to the judgment that ascribed the death of an enlisted military for non-Hodgkin's lymphoma to the mandatory multiple vaccination schedule administered to the soldier during his military service [5]. A careful analysis of nearly 1800 papers selected on PubMed according to the key words "lymphoproliferative disorders and vaccinations" retrieved only few anecdotal, not confirmed, reports of increased lymphoproliferation risk following influenza [6–7] and tuberculosis [Bacillus Calmette-Guérin (BCG)] [8–9] vaccines, in addition to the description of cutaneous pseudo-lymphoma by hepatitis B and A vaccines [10]. Conversely, influenza and BCG vaccinations have even been associated with a protective effect against lymphomas [11–12]. The significant increase of monoclonal gammopathies observed in individuals who had received multiple, even experimental, vaccinations decades before in comparison to controls is quite inexplicable. In fact, such an increase was not correlated with the type or amount of received vaccines, nor was it present on the occasion of the last check, thirty years before and twenty-five years after the end of vaccine stimulation [13]. More recently, vaccine adjuvants have been suspected as possible inducer of autoimmunity and lymphoma, as a presumed consequence of chronic inflammation due to immune system stimulation [14–15]. Moreover, a vaccine/adjuvant-driven evolution to lymphoproliferative disorders has even been observed in animals [16–20]. In healthy people, vaccine administration has occasionally been related to the occurrence of side effects, as reported in the probably most susceptible population of premature newborns [21] or in adults as kidney pathology rarely observed soon after vaccination with H1N1 influenza vaccine [22] and of autoimmune diseases, such as Guillain-Barré syndrome, immune thrombocytopenic purpura and myopericarditis, after swine flu (1976), measles-mumps-rubella and smallpox vaccinations, respectively [23,24]. Actually, in genetically predisposed subjects, the vaccination may rarely trigger an autoimmune reaction, through the mechanisms of molecular mimicry, bystander activation [24,25], epitope spreading or polyclonal activation [15].

The Centers for Disease Control and Prevention in 1998 have identified some pathological forms exclusive of the military returned from war campaigns in some war scenarios, such as the Multi-Symptomatic Syndrome, defined by fatigue and post-traumatic stress reaction [26]. In veterans of the first Gulf War the Multi-Symptomatic Syndrome has been linked to immune dysfunction, characterized by Th1 activation and IL10 secretion by memory cells [27]. Actually, some authors have suggested that immunological (concurrent vaccinations), accompanied by emotional (the mission to war theater) stress, could have contributed to cause an immunological imbalance, able to induce a state of ill health in veterans of the Gulf War [28,29]. This hypothesis has not been confirmed and more recently has been downgraded by the same research group [30]. Moreover, in the case of anthrax vaccination, Mahan et al. showed significant differences, in terms of health status, between the group of subjects who did report having received anthrax vaccination and those who did not. However, when the authors compared the group who self-reported and the veterans for whom vaccination records were available, these differences disappeared [31].

This background considered, the current study, based on clinical, molecular, and immunological analysis of two military groups, one operating in Italy and the other in Lebanon, was designed as an active surveillance for possible post-vaccine onset of clinical and/or laboratory autoimmunity/lymphoproliferation during a nine-month-long period.

## 2. Materials and methods

### 2.1. Study population

From September 2012 to June 2014 two groups of Italian military personnel, the first represented by newly-recruited students of military schools residing in Italy for at least 3 years and the other by soldiers operating abroad (Lebanon) for nine months, were enrolled. Exclusion criteria were: pregnancy, immunodepression, vaccine hypersensitivity.

The study was approved by the Italian Ministry of Defense ethical committee in July 2011, and registered in clinicaltrials.gov in 2012 with the identifier NCT01807780.

### 2.2. Vaccination schedule

At enrollment, informed consent and medical history of all individuals were collected. The vaccination schedule was personalized on the basis of information on history of infectious diseases and already received vaccinations; moreover, the vaccination schedule was even tailored on type of employment, either national or abroad. Administered vaccines were the following: tetanus/diphtheria (Td, DifTetAll-Novartis Vaccines and Diagnostics, Siena, Italy), inactivated polio (Imovax polio-Sanofi Pasteur MSD SpA, Roma, Italy), measles/mumps/rubella (MMR, Priorix -GlaxoSmithKline SpA, Verona, Italy), chickenpox (Varivax-Sanofi Pasteur MSD SpA, Roma, Italy) polysaccharide tetravalent (A, C, $W_{135}$,Y) meningococcal meningitis (Mencevax-PziferSrl, Latina, Italy), hepatitis A (HA, Epaxal-Crucell Italy Srl, Baranzate, Italy), hepatitis B (HB, Engerix B-GlaxoSmithKline SpA, Verona, Italy), influenza (Fluad-Seqirus Srl, Siena, Italy), and typhoid (Vivotif Berna-PaxVax Ltd., Birmingham, UK). Vaccines were generally administered the same day (in different arms), but in a few cases up to two weeks apart. However, a considerably lower number of subjects than expected were immunized with the aforementioned vaccines, as a consequence of previous vaccination records and history of infectious diseases.

### 2.3. Safety

Blood samples were collected before (time 0 [T0]) and one and nine months after vaccination (time 1 [T1] and time 2 [T2]) in group 1; at T0 and T2, following mission to Lebanon, in group 2.

Vaccine safety was examined by active monitoring of adverse events (at vaccination each subject was provided with a card to record any possible adverse events [local or systemic reaction, mild or severe]) and by the evaluation of the peripheral blood cell count, serum protein electrophoresis and serum immunoglobulins (Ig), to monitor the possible onset of signs suggestive of lymphoproliferative disorders. Moreover, the search for autoantibodies (anti-nuclear [ANA], anti-double-stranded DNA [anti-dsDNA], anti-extractable nuclear antigens [anti-ENAs], anti-phospholipid [APL], anti-neutrophil cytoplasmic antibodies [ANCA] and rheumatoid factor [RF]), was performed.

In 12/172 (7%) group 1 subjects, who had received ≥4 vaccinations, the presence of self-antigens recognized by specific antibodies was studied by microarray analysis at T0, T1 and T2. In 29/172 (17%) group 1 subjects, including those studied by microarray analysis, the possible cellular response to apoptotic self-epitopes, as an equivalent of cellular autoimmunity, was even investigated [32].