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Research Paper

Elevated Neopterin Levels Predict Early Death in Older Hip-fracture Patients

Martin Larsen [a], Charles Bayard [a], Hélène Lepetitcorps [a], Judith Cohen-Bittan [b], Victor Appay [a,c], Jacques Boddaert [b,c], Delphine Sauce [a,c,*]

[a] INSERM, U1135, Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), F-75013 Paris, France
[b] AP-HP, Service de gériatrie, Hôpital Pitié-Salpêtrière, F-75013 Paris, France
[c] Sorbonne Universités, UPMC Univ Paris 06, DHU FAST, CR7, F-75013 Paris, France

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ABSTRACT

Our society faces a major challenge concerning management of the health and socio-economic burden caused by acute physical stress in the older population (+75 years). In particular, hip-fracture surgery (HFS) represents a major health care preoccupation, affecting 1.6 million patients worldwide, resulting in a significant drop in life quality and autonomy. The trauma is associated with 20–30% one-year mortality in the elderly. In the present study, we aim to identify factors, which influence and/or predict the outcome of elderly hip- fracture patients (HFP) post-surgery.

Our objective was to identify biomarkers with a prognostic capacity of one-year mortality. We employed an observational cohort of HFP (n = 60) followed-up longitudinally during the first year post fracture. Clinical and biological data (n = 136), collected at arrival to hospital, were then compared to healthy controls (n = 42) and analyzed using a regularized logistic regression model with lasso penalty followed by 10-fold cross-validation of variables.

We show that plasmatic neopterin levels, a molecule released by IFN-γ-activated macrophages, is predictive of mortality in HFP (ROC-AUC = 0.859). Moreover, neopterin measured at arrival to the hospital correlated negatively with the time of survival after HFS.

Neopterin therefore represents a biomarker, which enables better follow-up of patients at risk of early death.

1. Introduction

Aging is characterized by a progressive decline of physical and mental performances. This geriatric syndrome, coined frailty, is increasing incrementally with advancing age, and more rapidly in older women and among those of lower socio-economic status. Frail older adults are at high risk of major adverse health outcomes, including disability, falls, institutionalization, hospitalization, and mortality. One of the complications of frail elderly is hip-fracture (HF).

HF is a common condition associated with a 20–30% one-year mortality in the elderly (Roche et al., 2005; Jennings and Boer, 1999). Indeed, HF patients belong to a vulnerable group of old people with comorbid diseases and a high risk of postoperative morbidity and mortality. The high incidence of HF in elderly (3%) raises an increased concern in a world with an aging population. Thus, the number of annual HFs worldwide is actually 1.6 million, but is expected to reach >4 million in 2050 (Gullberg et al., 1997), and has an estimated annual cost > $8 billion/year for inpatients only (Ray et al., 1997). This may reach $14 billion/year in US if including the costs that can be incurred by rehabilitation/rehospitalization over a period of one year after HFS. Consequently, a major challenge is the management of the health and socio-economic burden caused by this acute physical stress in the older population (+75 years). According to Bouchon's definition (Bouchon, 1984), HF represents an acute event that can participate to the accelerated decline of health in the elderly.

Factors associated with the high morbidity burden post HFS commonly include older age and cardiorespiratory comorbidities as shown in the ESCORTE study analyzing the outcomes after HFS in a large cohort of French patients (Rosencher et al., 2005). Other clinical and biological factors have been associated with mortality (Laulund et al., 2012), including gender, comorbidities (Roche et al., 2005), post-operative complications (Beloosesky et al., 2007), low albumin (Pimlott et al., 2011), high creatinine (Seyedi et al., 2015), increased post-operative troponin (Chong et al., 2009), elevated procalcitonin post-surgery (Vallet et al., 2017). Moreover, hip-fracture is known to be associated with major elevation of inflammatory cytokines, such as IL-6 or TNF-α (Briza et al., 2002; Sedlar et al., 2010, 2008; Sun et al., 2011), suggesting that inflammation may play a major role in the patients' outcome.

Abbreviations: HF, hip-fracture; HFS, hip-fracture surgery; HFPs, hip-fracture patients; PBMCs, peripheral blood mononuclear cells.

* Corresponding author at: Delphine Sauce, Infections and Immunity, INSERM U1135, Hôpital Pitié-Salpêtrière, 75013 Paris, France.
E-mail address: delphine.sauce@upmc.fr (D. Sauce).

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Neopterin (1′, 2′, 3′-D-erythro-trihydroxypropylpterin) belongs to the group of pteridines and is reported to be associated with activation of cell-mediated immunity, which is tightly associated with hyper-inflammation. Neopterin is derived in vivo from guanosine triphosphate (GTP) through a reaction catalyzed by the enzyme GTP-cyclohydrolase-I (GCH I), which is expressed by activated monocytes, macrophages, dendritic cells and endothelial cells upon stimulation by IFN-γ (Fuchs et al., 1993; Murr et al., 2002).

Here we assess how the immune system of hip-fracture patients (HFPs) copes with acute stress. Indeed, we hypothesized that biological factors associated with host immunity may predict successful recovery in older patients, defined by resilience and long-term survival after surgery. We found that increased neopterin plasmatic levels pre-surgery are predictive of non-survival post hip-fracture and may even predict time of survival after HFS. Therefore, elevated neopterin levels reflect an elevated and seemingly unregulated immune activation taking place in acute HFPs. In conclusion, neopterin measures constitute a prognostic biomarker for HFPs, which may aid to optimize patient's management and reduce public health costs.

2. Materials and Methods

2.1. Study Subjects

Participants were selected according to rigorous inclusion criteria: they were aged 75 years and older, free of medication and diseases affecting the immune system (e.g., cancers, autoimmune disorders), absence of prior physical disabilities and, absence of cognitive disorders. A total of 102 elderly were included for this case-control design study with half being healthy controls ($n = 42$) and another half suffering from hip-fracture (HF; $n = 60$; Fig. 1). This last group was admitted to the Department of Emergency and orthopedic surgery at Pitié-Salpetrière Hospital (Paris, France) for a fracture of the hip. Follow-up was performed up to one –year post surgery.

Heparinized blood samples collected from HF patients before surgery were set as the reference point (day of arrival to hospital). For each individual, PBMCs (isolated by density gradient centrifugation) and plasma were cryopreserved until use. Experiments were performed without knowing clinical outcome which was defined at later timepoint.

2.2. Power Analysis

The required HFP cohort size was estimated from a power analysis for a biomarker with a high Cohen's d effect size of 0.8, type-1 error ($\alpha = 0.05$) and type-2 error ($\beta = 0.2$) given that 1/3 of the cohort would finally display the clinical outcome, death. The latter value was obtained from literature (Roche et al., 2005; Jennings and Boer, 1999). The HFP cohort size under these conditions is 60. Power analysis was performed using the software package G*Power (http://www.gpower.hhu.de/en.html).

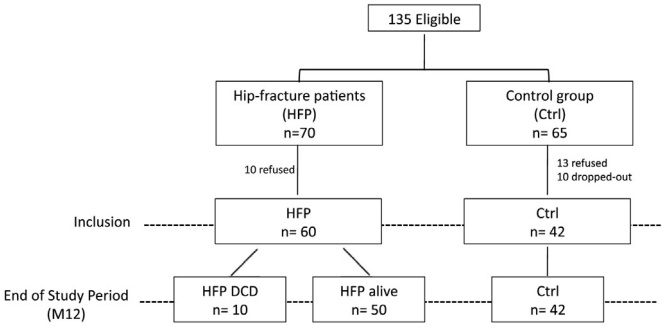

Fig. 1. Design of the study.

2.3. Data Collection

Data collected prospectively included age, sex, previous medical history, fracture type, surgical treatment, and duration of surgery (Boddaert et al., 2014a). Associated comorbidities were carefully reviewed, and their severity was assessed using the Cumulative Illness Rating scale (CIRS), a validated scale for elderly population, in which concurring medical conditions are weighted from 0 to 4 in 13 main systems (Linn et al., 1968). According to Zekry and colleagues, the CIRS improved hospital discharge planning for elderly patients with acute disease (Zekry et al., 2012). Functional status was assessed by the Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL) scales. C-reactive protein (CRP) was measured by automatic laser nephelometry (BN 100 analyzer; Boehring Dade, Marburg, Germany), normal values were <5 mg/L, and the coefficient of variation of the measurement was <5%. Pre- and post-operative hemoglobin level, post-operative serum creatinine, estimated creatinine clearance using the Cockroft formula, and serum albumin level were recorded. Chronic renal failure was defined as an estimated Cockcroft creatinine clearance ≤60 mL/min, malnutrition was defined as an albumin value ≤35 g/L and procalcitonin concentrations were analyzed using a sandwich immunoassay based on time resolved amplified cryptate emission (TRACE) measurement (Kryptor analyzer; B.R.A.H.M.S. Thermofisher, Hennigsdorf, Germany), as previously reported (Boddaert et al., 2014a; Vallet et al., 2017).

2.4. Ethics Statement

Clinical investigation has been conducted according to Declaration of Helsinki principles. All participants were recruited in Pitié Salpétrière Hospital (Paris) and provided written informed consent. The study was approved by the "Comité de Protection des Personnes" of the Pitié Salpétrière Hospital, Paris.

2.5. Flow Cytometry Analysis

From fresh blood, absolute counts (cells per microliter) were determined using CYTO-STAT tetraCHROME kits on a FC500 cytometer (Beckman Coulter) and analyzed with Flow-Count Single Platform Method (Beckman Coulter). Directly conjugated and unconjugated antibodies were obtained from the following vendors: BD Biosciences (San Jose, CA): CD4 (HV500), CD8 (APC-Cy7), CD16 (APC-H7), CD56 (PE-Cy7); Beckman Coulter (Pasadena, CA): CD3 (ECD), CD45 (KO), NKG2A (APC), CD45RA (ECD); BioLegend (San Diego, CA): CD57 (PB), CD3 (BV650); CD8 (BV650), CD27 (AF700); R&D systems (Abingdon, UK): NKG2C (PE). Staining for cell surface markers was performed with standard method as previously described (Bayard et al., 2016). Cells were analyzed on a Fortessa flow cytometer (Becton Dickinson). Data were analyzed using FlowJo v8.2 (Tree Star, Inc) and DIVA softwares (BD Biosciences). Exhaustive phenotypic analysis of NK cells was conducted with the "FunkyCells ToolBox" software (www.FunkyCells.com).

2.6. Plasmatic Measurements

Levels of soluble CD14s (sCD14) and neopterin were determined by commercially available Elisa kits (Quantikine R&D systems and alpha diagnostic, respectively). Measures of the soluble factors IFN-inducible protein 10 (IP-10), and IL-6 were performed with the use of multiplex bead immunoassays (Biosource) and Luminex instrument. Ultrasensitive detections of IL-1β, IFN-α were obtained with Simoa technology (Quanterix). All experiments were performed by following manufacturer's instructions.

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