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Research Paper

Immunometabolic and Lipidomic Markers Associated With the Frailty Index and Quality of Life in Aging HIV+ Men on Antiretroviral Therapy

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ABSTRACT

Chronic immune activation persists despite antiretroviral therapy (ART) in HIV+ individuals and underpins an increased risk of age-related co-morbidities. We assessed the Frailty Index in older HIV+ Australian men on ART. Immunometabolic markers on monocytes and T cells were analyzed using flow cytometry, plasma innate immune activation markers by ELISA, and lipidomic profiling by mass spectrometry. The study population consisted of 80 HIV+ men with a median age of 59 (IQR, 56–65), and most had an undetectable viral load (92%). 24% were frail, and 76% were non-frail. Frailty was associated with elevated Glucose transporter-1 (Glut1) expression on the total monocytes ($p = 0.04$), increased plasma levels of innate immune activation marker sCD163 (OR, 4.8; CI 1.4–15.9, $p = 0.01$), phosphatidylethanolamine PE(36:3) (OR, 5.1; CI 1.7–15.5, $p = 0.004$) and triacylglycerol TG(16:1_18:1_18:1) (OR, 3.4; CI 1.3–9.2, $p = 0.02$), but decreased expression of GM3 ganglioside, GM3(d18:1/18:0) (OR, 0.1; CI 0.0–0.6, $p = 0.01$) and monohexosylceramide HexCerd(d18:1/22:0) (OR, 0.1; CI 0.0–0.5, $p = 0.004$). There is a strong inverse correlation between quality of life and the concentration of PE(36:3) ($\rho = -0.33$, $p = 0.004$) and PE(36:4) ($\rho = -0.37$, $p = 0.001$). These data suggest that frailty is associated with increased innate immune activation and abnormal lipidomic profile. These markers should be investigated in larger, longitudinal studies to determine their potential as biomarkers for frailty.

1. Introduction

As the median age of people living with HIV increases globally (UNAIDS, 2013), there is a growing need to identify and manage the compounded effects of HIV and aging. In geriatrics, it is generally accepted that chronological age may not accurately reflect physiologic reserve. Frailty conceptualizes a "biologic age", describing an overall physiologic vulnerability to stressors. Frailty is not only a predictor of adverse outcomes during aging, it has also been used to individualize treatment goals for chronic comorbidities to improve health outcomes (Blodgett et al., 2016; Strain et al., 2013). Chronically infected and long-term treated HIV+ people have a heightened risk, and possibly premature onset of, a number of non-AIDS comorbidities including cardiovascular disease, non-AIDS cancers, renal and bone disease, liver disease as well as frailty (Deeks, 2011).

The clinical definition of frailty remains controversial, but one validated measure is the Frailty Index, where frailty is the result of an accumulation of health deficits over time, and the severity of frailty can predict adverse health outcomes (Rockwood and Mitnitski, 2007).

In the general population, frailty has been shown to be associated with activation of the innate immune system (Collerton et al., 2012). Similarly, HIV infection is associated with biomarkers of innate immune system activation. sCD14 remains elevated despite effective antiretroviral therapy, and has also been identified as a potential biomarker for non-AIDS mortality risk in HIV+ people (Méndez-Lagares et al., 2013; O'halloran et al., 2015). Elevated levels of other innate immune activation markers including soluble CD163 (sCD163), which is shed from macrophages via proteolytic cleavage by the sheddase ADAM-17 following an inflammatory stimulus (Etzerodt et al., 2010), and thus is also considered an inflammatory marker, have also been linked with both HIV infection and aging (Hearps et al., 2012; O'halloran et al.,

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2015). Recognition of a major role of the innate immune system in driving age-related comorbidities via chronic inflammation and senescent change marks a shift from the traditional focus on adaptive immune dysfunction that is largely responsible for $CD4^+$ T cell depletion in untreated HIV infection (Hearps et al., 2014).

Immunometabolism is a rapidly evolving area providing insights into how metabolic changes in immune cells impact immunity and drives inflammatory diseases. Overwhelming evidence indicate specific metabolic demands of immune cells during an infection. In HIV+ individuals, increased glucose metabolic activity in inflammatory CD16+ monocyte subsets was associated with inflammation, and this metabolic activity was not normalized in patients on antiretroviral therapy (Anzinger et al., 2014; Palmer et al., 2014a). This metabolic activity is controlled by the mitochondria and may be perturbed by HIV itself or ART toxicity. The immunometabolic concepts of this study present potential prognostic and therapeutic opportunities. In particular, glucose transporter 1 (Glut1), the major glucose transporter on inflammatory monocytes and macrophages, is overexpressed on inflammatory monocytes/macrophages to maintain activation and produce pro-inflammatory cytokines (Palmer et al., 2014a; Freemerman et al., 2014).

HIV infection and antiretroviral therapy are both associated with significant disturbances in systemic metabolic regulation, including lipids, which play a central role in the host immune response, as previously reviewed (Willig and Overton, 2016). While dyslipidemia is a recognized risk factor for age-related diseases, such as cardiovascular disease, only a handful of studies have investigated the plasma lipid profiles in HIV infection, and these have focused on cardiovascular risk prediction (Wong et al., 2014; Low et al., 2016). There is a paucity of data reflecting the association between dysregulated lipid metabolism with frailty.

Thus, we conducted a comprehensive profiling of key plasma lipid species in older HIV+ men to uncover previously unrecognized lipids that may play a role in the immunopathogenesis of frailty. We sought to investigate the relationship between frailty, innate immune activation, and the immunometabolic and lipidomic profiles of older men with HIV infection.

2. Materials and Methods

2.1. Study Population

Participants were recruited voluntarily between March and August 2015 from the HIV outpatient clinic and Fairfield House, a sub-acute inpatient facility, at The Alfred hospital, a tertiary care facility in Melbourne, Australia. Individuals were eligible if they were living with HIV, of male sex, aged fifty years or older, able to participate in the study assessments, and were taking combination antiretroviral therapy for at least six months. Approval for this study was obtained from the Alfred Health Human Research Ethics Committee and the Monash University Human Research Ethics Committee (Project No. 41/15), with a signed informed consent provided by all volunteers.

2.2. Clinical Assessments

All participants completed a single study visit comprising a standardized interview, clinical frailty assessments, and review of available medical records. Time since HIV diagnosis, previous AIDS diagnoses, nadir $CD4^+$ T-cell count, antiretroviral drug history, and anthropometric data from the prior 12 months were collected from medical records. Current comorbidities and medications were self-reported and confirmed by medical records. These included non-AIDS cancers, liver disease, mental health illnesses, arrhythmias, congestive heart failure, venous thromboembolism, hypertension, ischemic heart disease, peripheral vascular disease, chronic respiratory disease, painful arthropathies, osteoporosis, rheumatoid arthritis, cerebrovascular disease, renal disease, neurological diseases, diabetes mellitus and thyroid disorders. Laboratory measures were obtained from blood tests performed on the day of assessment, or from the medical record within six months of study enrollment.

2.3. Frailty Assessment

Frailty was assessed using the Frailty Index. The Frailty Index implemented in this study was composed of 37 age-related health variables, previously published, with minor adaptations (Searle et al., 2008). Modifications were the removal of peak flow, shoulder strength and rapid pace due to lack of feasibility of performing these in the clinic. We also added urinary incontinence as a variable, as this was an important functional indicator, and met the five criteria for inclusion into the Frailty Index as a variable (Searle et al., 2008). The number of reported deficits was divided by the number of assessed variables to calculate a final ratio score for each individual ranging from 0 to 1, with 1 indicating the worst frailty. Comorbidities were assessed as "present" if self-reported and verified within medical records, and "suspected" if detected during the study visit, but with no formal diagnosis or not receiving treatment for the condition. Participants with a Frailty Index score > 0.25 were considered "frail" and ≤0.25 "non-frail" (Rockwood et al., 2007).

2.4. Quality of Life

Quality of life was measured using the RAND-36 measure of health-related quality of life, a 36-item survey used commonly in the HIV+ population. Quality of life was calculated as a score from 0 to 100, as previously described (Hays and Morales, 2001). Higher values indicate higher self-reported quality of life.

2.5. Peripheral Blood Mononuclear Cell (PBMC) Preparation

Thirty milliliters of whole blood was collected in EDTA anticoagulant and processed within 2 h of collection to minimize cell activation or cytokine production. PBMCs were purified from whole blood using Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden) or Lymphoprep (Axis-Shield, Oslo, Rodel, Norway). PBMCs were cryopreserved in 10% dimethyl sulfoxide in RPMI medium as previously described (Palmer et al., 2014b).

2.6. Glucose Transporter-1 Expression and Mitochondrial Function

Glycolytic activation was analyzed by quantifying Glut1 surface expression on cryopreserved PBMCs using flow cytometry as previously described (Palmer et al., 2014a). Briefly, PBMCs were stained with fluorescently labeled anti-CD3/CD14/CD16 monoclonal antibodies. Cell surface Glut1 was detected using Glut1 antibody [MAB1418 clone (R&D Systems, Minneapolis, Minnesota, USA)] conjugated with FITC. Cells were stained and acquired as previously described (Palmer et al., 2014a).

To measure mitochondrial membrane potential, PBMCs were stained as above and pellets resuspended in 100 μL of 1 × PBS containing 2 μL of 1 μM $DiOC_6(3)$, a fluorescent lipophilic dye that is selective for mitochondria. Cells were incubated in the dark at 37 °C for 30 min, washed twice with 0.5% BSA in 1 × PBS, and resuspended in 300 μL of 1 × PBS.

Samples were analyzed immediately by flow cytometry or stored in the dark at 4 °C for up to 30 min before data acquisition.

2.7. T-lymphocyte Immune Activation

To measure T cell activation, PBMCs were stained with fluorescently labeled anti-CD3-FITC/CD4-PerCPCy5.5/CD8-APCH7/HLA-DR-PE-Cy7/CD38-PE monoclonal antibodies as previously described (Palmer et al., 2014b).

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