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Role of senescence marker p16^{INK4a} measured in peripheral blood T-lymphocytes in predicting length of hospital stay after coronary artery bypass surgery in older adults

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ABSTRACT

Adults older than 65 years undergo more than 120,000 coronary artery bypass (CAB) procedures each year in the United States. Chronological age alone, though commonly used in prediction models of outcomes after CAB, does not alone reflect variability in aging process; thus, the risk of complications in older adults. We performed a prospective study to evaluate a relationship between senescence marker p16^{INK4a} expression in peripheral blood T-lymphocytes (p16 levels in PBTLs) with aging and with perioperative outcomes in older CAB patients. We included 55 patients age 55 and older, who underwent CAB in Johns Hopkins Hospital between September 1st, 2010 and March 25th, 2013. Demographic, clinical and laboratory data following outline of the Society of Thoracic Surgeons data collection form was collected, and p16 mRNA levels in PBTLs were measured using TaqMan® qRT-PCR. Associations between p16 mRNA levels in PBTLs with length of hospital stay, frailty status, p16 protein levels in the aortic and left internal mammary artery tissue, cerebral oxygen saturation, and augmentation index as a measure of vascular stiffness were measured using regression analyses. Length of hospital stay was the primary outcome of interest, and major organ morbidity, mortality, and discharge to a skilled nursing facility were secondary outcomes. In secondary analysis, we evaluated associations between p16 mRNA levels in PBTLs and interleukin-6 levels using regression analyses. Median age of enrolled patients was 63.5 years (range 56–81 years), they were predominantly male (74.55%), of Caucasian descent (85.45%). Median $\log_2$(p16 levels in PBTLs) were 4.71 (range 1.10–6.82). P16 levels in PBTLs were significantly associated with chronological age (mean difference 0.06 for each year increase in age, 95% CI 0.01–0.11) and interleukin 6 levels (mean difference 0.09 for each pg/ml increase in IL-6 levels, 95% CI 0.01–0.18). There were no significant associations with frailty status, augmentation index, cerebral oxygenation and p16 protein levels in blood vessels. Increasing p16 levels in PBTLs did not predict length of stay in the hospital (HR 1.10, 95% CI 0.87–1.40) or intensive care unit (HR 1.02, 95% CI 0.79–1.32). Additional evaluation of p16 levels in PBTLs as predictor of perioperative outcomes is required and should include additional markers of immune system aging as well as different outcomes after CAB in addition to length of hospital stay.

1. Introduction

Coronary artery bypass (CAB) is among the most commonly performed surgical procedures in older adults, involving over 120,000 patients older than 65 years in the United States alone (Go et al., 2014). Models which predict length of hospital and intensive care unit (ICU) stay after cardiac surgery commonly incorporate chronological age (Go et al., 2014; Janssen et al., 2004; Ghotkar et al., 2006; Messaoudi et al., 2009). Unfortunately, chronological age does not accurately predict an individual's aging process and decline in physiologic reserve that can be accelerated or retarded in response to environmental

Abbreviations: AI, Augmentation index; BMI, Body mass index; CAB, Coronary artery bypass; CAD, Coronary artery disease; CI, Confidence interval; CPB, Cardiopulmonary bypass; IL-6, Interleukin 6; ICU, Intensive care unit; LIMA, Left internal mammary artery; OAIC, Older Americans Independence Center; P16 levels in PBTLs, p16^{INK4a} levels in peripheral blood T-lymphocytes; RNA, Ribonucleic acid; ScO_2, Cerebral oxygen saturation.

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exposures, host experiences, and genetics (Mitnitski et al., 2002; Fried et al., 1998); therefore, identification and utilization of 'aging biomarkers' that accurately measure patients' physiologic reserve is an important clinical need.

Aging is characterized by a reduction in the regenerative capacity of many tissues, and the accumulation of senescent cells appears to broadly contribute to tissue aging (Wagers and Conboy, 2005; Sharpless and DePinho, 2007; Janzen et al., 2006; Rodier and Campisi, 2011). Cellular senescence is an irreversible growth arrest (Hayflick, 1965) that occurs in response to a variety of noxious stimuli (e.g., DNA damage, telomere shortening, oxidative stress, and epigenetic damage) (Wright and Shay, 2002; Kirkwood, 2005; Kuilman et al., 2010). Senescent cells do not replicate, which impairs tissue homeostasis (Drummond-Barbosa, 2008), and secrete pro-inflammatory cytokines (Coppe et al., 2010), associated with age-associated phenotypes such as sarcopenia, immune dysfunction and delayed wound healing (Ashcroft et al., 2002). Therefore, measuring the accumulation of senescent cells in vivo has been suggested to provide a means of measuring the 'molecular age' of the organism.

The $p16^{INK4a}$ senescence marker has been suggested to serve as a biomarker of aging and predictor of physiologic reserve (Sharpless and DePinho, 2007; Dimri, 2004). Expression of $p16^{INK4a}$ is not detected in young cells, but is potently activated by stress factors that promote cellular senescence (Brenner et al., 1998; te Poele et al., 2002; Kim and Sharpless, 2006; Song et al., 2010). Senescent cells remain in tissues indefinitely; therefore, accumulation of $p16^{INK4a}$ expression reflects the accumulation of senescent cells with aging and as such, expression of $p16^{INK4a}$ is intrinsic to the aging process. Expression of the $p16^{INK4a}$ transcript is highly dynamic, increasing exponentially with chronologic age in all mammalian species tested to date (Zindy et al., 1997; Melk et al., 2003; Krishnamurthy et al., 2004). The ability of $p16^{INK4a}$ in human kidney allograft biopsies predicts kidney function based on creatinine level at 6 month and 1 year post-transplant and performs better than telomere length (McGlynn et al., 2009; Koppelstaetter et al., 2008; Gingell-Littlejohn et al., 2013). Interestingly, in humans, expression of $p16^{INK4a}$ in peripheral blood T-lymphocytes (PBTL) changes >10-fold (Liu et al., 2009) throughout lifespan. Additionally, $p16^{INK4a}$ expression in PBTL shows a much stronger correlation with chronologic age than do other aging biomarkers (Liu et al., 2009) (r^2 0.6–0.7 for $p16^{INK4a}$, $r^2 < 0.2$ for leukocyte telomere length or IL-6). While the role of $p16^{INK4a}$ as a molecular age biomarker is becoming established, the clinical utility of measuring $p16^{INK4a}$ levels to predict clinically-relevant outcomes is not well described, though promising as demonstrated by previous work in kidney transplantation.

In this prospective pilot study of older adults undergoing CAB procedure, we asked two questions: can $p16^{INK4a}$ expression serve as a biomarker of aging in this patient population; and can $p16^{INK4a}$ levels predict poor clinical outcomes. We hypothesized that older adults have higher levels of $p16^{INK4a}$, and that $p16^{INK4a}$ mRNA levels in PBTLs correlates with other markers of aging including frailty, p16 protein levels in vascular walls, cerebral oxygen saturation and measures of vascular stiffness. We further hypothesized that patients with higher $p16^{INK4a}$ mRNA levels in PBTLs have a slower recovery, and therefore are more likely to have increased length of hospital stay, compared to patients with lower $p16^{INK4a}$ mRNA levels in PBTLs.

2. Material and methods

2.1. Institutional review board

The Johns Hopkins Medicine Institutional Review Board (IRB) approved the study NA_00032660.

2.2. Study design and setting

We have conducted a prospective study of older adults undergoing CAB procedure in urban, academic, tertiary care hospital (Johns Hopkins Hospital). All participants were recruited between September 1st, 2010 and March 25th, 2013 and followed for 30 days after their surgical procedure.

2.3. Subjects

We have included all patients 55 years of age and older undergoing primary elective or urgent CAB surgery. We have excluded patients requiring emergency or salvage CAB surgery, being reoperated, undergoing combined, aortic or valvular surgical procedures, primary ventricular assist device implantation, having any acute illness other than coronary artery disease, or requiring preoperative inotropic or vasoactive medications. We adjusted our inclusion/exclusion criteria after initial slow recruitment rate as follows: minimal age of participants was decreased from 65 to 55, and exclusions such as ejection fraction less than 40% and participation in other research protocols were removed.

All patients scheduled to undergo CAB surgical procedure were identified through the operating room schedule. We then screened electronic medical records of all identified patients for inclusion/exclusion criteria. Eligible patients were approached by the research personnel either in the pre-operative clinic or at the bedside in the hospital at least one day prior to the scheduled surgical procedure. The patient's surgical attending physician was notified of the patient's enrollment into the study. All study participants provided informed consent on their participation in the study.

2.4. Study procedures

Upon enrollment we collected the following participant's information: demographic characteristics of age, gender, race; anthropomorphic characteristics of weight, height and body mass index (BMI); smoking, exercise and alcohol consumption; organ system diseases through self-report and chart review; current medications; functional capacity including basic and instrumental activities of daily living (ADLs (Katz et al., 1970) and IADLs (Lawton and Brody, 1969)), ability to drive; laboratory data reflecting major organ system function (white cell count, hemoglobin level, platelets, blood urea nitrogen, creatinine, albumin, alkaline phosphatase, total bilirubin and calcium levels, international normalized ratio), heart rate and myocardial ejection fraction. Comorbidities burden was summarized by Charlson index (Charlson et al., 1987). We then performed following measurements: frailty assessment, vascular stiffness, $p16^{INK4a}$ levels in PBTLs and vascular wall, interleukin 6 (IL-6) levels in serum, and ScO_2.

2.4.1. Frailty Assessment

Frailty assessment was performed pre-operatively following methodology previously described (Fried et al., 2001). Briefly, frailty was measured using a previously validated scoring system evaluating 5 domains: (1) shrinking defined as unintentional weight loss of 10 lb or more in the last year; (2) weakness determined by a grip-strength test and adjusted for gender and body mass index (BMI); (3) exhaustion as measured by two questions from the modified 10-item Center for Epidemiological Studies-Depression scale; (4) low physical activity as measured by a version of the Minnesota Leisure Time Activities Questionnaire (Taylor et al., 1978); and (5) slowed walking speed as measured by averaging three trials of walking 15 ft at a normal pace. Each domain yielded a dichotomous score of 0 or 1. Patients were categorized based on their total score into frail (total score 3–5), prefrail (total score 1–2), and nonfrail (total score 0).

2.4.2. Vascular stiffness

Vascular stiffness was assessed preoperatively by measuring pulse pressure (PP) based on oscillometric blood pressure measurement averaged over 3 repeated measurements and augmentation index (AI) obtained non-invasively by applanation tonometry (SPT-301, Millar, Inc., Houston, TX) from the radial artery. AI was calculated using a software