Urine Desmosine as a Marker of Lung Injury Following Total Knee Arthroplasty. A Pilot Study

Stavros G. Memtsoudis, MD, PhD · Barry Starcher, PhD · Alejandro González Della Valle, MD · Yan Ma, PhD · Kethy Jules-Elysee, MD · Thomas P. Sculco, MD

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Abstract Lung injury following total knee arthroplasty (TKA) may occur secondary to embolization of bone debris, fat, and cement. Clinically relevant respiratory failure is rare and is therefore difficult to study. To facilitate future investigations on this subject, we evaluated the utility of the elastin breakdown product desmosine as a potential marker of lung injury during TKA surgery. The goals of this

study were to answer (1) if desmosine levels would increase in response to the perioperative insults in patients undergoing TKA and (2) if this increase would differ among unilateral and bilateral TKA procedures. Twenty consecutive patients (ten unilateral and ten bilateral TKAs) were enrolled. Urine samples were collected before surgery and at 1 and 3 days postoperatively and analyzed for levels of desmosine using a validated radioimmunoassay. Baseline desmosine/creatinine ratios were higher in the unilateral as compared to the bilateral TKA group ($p=0.003$). Tourniquet times, intra-operative estimated blood loss, and transfusion requirements among bilateral TKA patients were significantly higher than those of unilateral TKA recipients. Desmosine levels increased in both groups, but the rise was significant only in the bilateral group. We detected a significant increase in urine desmosine levels associated with bilateral but not unilateral TKA surgery. In the context of previous studies, our findings suggest that desmosine may be a marker of postoperative lung injury. Further research is warranted for validation and correlation of desmosine levels to clinical markers and various degrees of lung injury.

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Each author certifies that his or her institution has approved the human protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research and that informed consent for participation in the study was obtained.

Level of evidence: Level III: Prognostic study

S. G. Memtsoudis, MD, PhD · K. Jules-Elysee, MD
Department of Anesthesiology,
Hospital for Special Surgery,
535 East 70th Street, New York, NY 10021, USA

A. González Della Valle, MD · T. P. Sculco, MD
Department of Orthopedic Surgery,
Hospital for Special Surgery,
535 East 70th Street, New York, NY 10021, USA

B. Starcher, PhD
Department of Biochemistry,
University of Texas Health Center, Tyler, TX, USA

Y. Ma, PhD
Department of Biostatistics,
Hospital for Special Surgery,
535 East 70th Street, New York, NY 10021, USA

S. G. Memtsoudis, MD, PhD (✉) · A. González Della Valle, MD · Y. Ma, PhD · T. P. Sculco, MD
Weill Cornell Medical College,
525 East 68th Street, New York, NY 10065, USA
e-mail: MemtsoudisS@hss.edu

Introduction

Lung injury after total knee arthroplasty (TKA) surgery may occur secondary to perisurgical events [1–6]. While the exact mechanism of lung injury associated with knee surgery remains unclear and may be multifactorial, it is likely secondary to embolization of bone, cement, and marrow debris. Analysis of bronchoalveolar specimens has linked the presence of lipid laden macrophages to possible embolization of fat and debris entering the blood stream during other orthopedic procedures involving instrumentation of bone and displacement of bone marrow [7]. This mechanism of lung injury is supported by echocardiographic studies that show embolic material entering the right heart in conjunction with knee surgery [1, 4].

In the vast majority of patients with adequate cardiopulmonary reserve, this phenomenon is of subclinical consequence. Symptomatic acute respiratory distress syndrome (ARDS)

rarely develops and the extent of lung injury is difficult to quantify. Consequently, researchers have resorted to surrogate indicators of pulmonary damage, including the measurement of pulmonary vascular resistance [5] and biomarkers of subclinical lung injury, such as angiotensin-converting enzyme activity [6]. These approaches are burdened by their invasiveness, lack of specificity, and difficulty in the retrieval and analysis of specimens.

Desmosine is a unique and stable breakdown product of elastin that is easily measurable in urine [8]. It has been proposed as a surrogate marker of lung injury in patients with cystic fibrosis [9], chronic obstructive pulmonary disease [10], and tobacco use [11] and elevated levels have recently been linked to increased mortality in ARDS patients [12].

During lung injury, elastin breakdown takes place secondary to exposure of lung tissue to proteases from neutrophils and macrophages [13]. Among the breakdown products are cross-linked desmosine and isodesmosine, which are unique to elastin, are extremely stable, and are excreted in the urine [13].

The utility of desmosine as a marker of lung injury in surgical patients, and especially patients undergoing orthopedic procedures, however, remains unstudied. In order to determine if this compound may be of use for the study of pulmonary damage in the setting of TKA surgery, we investigated (1) if desmosine levels would increase in response to the perioperative insults in patients undergoing TKA and (2) if this increase would differ among patients undergoing unilateral and bilateral TKA (BTKA and UTKA) procedures.

Materials and methods

After approval by the institutional review board, we enrolled 20 consecutive patients who underwent either primary unilateral ($N=10$) or bilateral ($N=10$) TKA for osteoarthritis between April and June of 2008. Operations were performed by a number of different surgeons (AD, TS, CC, DA, MA, SH, MF, RW, AK, HR). Patients with renal insufficiency were excluded.

Patients undergoing BTKA were selected by using the Hospital for Special Surgery criteria, which excludes patients over the age of 75, and patients with significant active cardiopulmonary disease [14]. Patient demographics (Table 1) and perioperative data were recorded. All 20 enrolled patients completed the study. Although no statistically

significant demographic differences were found among the two groups, patients in the unilateral group were more likely female and older when compared with the bilateral TKA group. On average, patients in both groups were overweight ($25 \leq \text{body mass index (BMI)} \leq 29.9$) and those in the BTKA group had a higher tendency toward obesity ($\text{BMI} \geq 30$).

Urine samples were collected at baseline (before incision), the first postoperative day, and the third postoperative day (in 24-h intervals) and analyzed for levels of desmosine using a previously validated radioimmunoassay [8, 13, 15]. Concomitant analysis of urine creatinine levels was performed to adjust for dilution as previously described [13]. Urine creatinine levels have been used as a common denominator based on the notion that the amount of creatinine in urine is constant in the absence of kidney disease. Thus, the concentration will vary based on dilution. In contrast, desmosine amounts will vary in the urine depending on the amount of elastin breakdown. Results are presented as picomole desmosine per milligram creatinine.

All procedures were performed under combined spinal and epidural anesthesia. All patients received unilateral or bilateral femoral nerve blocks for postoperative pain control and were monitored according to standard guidelines of the American Society of Anesthesiologists. In addition, blood pressures were monitored invasively with a radial artery catheter. Central venous pressure was monitored in patients receiving bilateral TKAs. Sedation was provided with midazolam and propofol. Fentanyl, diazepam, and ketamine were added as deemed necessary by the attending anesthesiologist. The surgical procedures were performed under tourniquet inflation to 250 mmHg after exsanguination of the extremity. All patients received a posterior stabilized total knee prosthesis. The tibial cut was performed using an extramedullary guide and the tibial canal was not invaded. The femoral work was done utilizing a long intramedullary guide. In order to minimize forcing the intramedullary content into the peripheral venous system, the femoral canal was thoroughly aspirated before insertion of the intramedullary rod that guided the femoral cuts and before impacting a bone plug that occluded the distal femoral opening. All components were fixed with acrylic cement. The patella was resurfaced in all cases. Bilateral TKAs were performed sequentially during the same anesthetic. All patients received vacuum drains that were removed on the first postoperative day. Postoperatively, patients were recovered in a monitored setting and transferred to the ward when deemed to be stable. Bilateral TKA recipients were observed in the recovery room overnight as per standard protocol.

We modeled the relationship of the desmosine level as a function of time (baseline, postoperative day 1, and postoperative day 3) using linear regression with inference based on the generalized estimating equation method [16]. Changes in urine desmosine levels over time were detected using appropriate linear contrasts. Continuous and discrete demographics variables at baseline were compared between the procedure types using *t* test and Fisher's exact test, respectively. A *p* value of <0.05 was considered significant. Results are shown as mean $\pm$ standard deviation.

Table 1 Patient demographics

Variable	BTKA	UTKA	<i>p</i> value
Age (years $\pm$ SD)	62 $\pm$ 11.4	70.5 $\pm$ 9.1	0.08
Gender (male/female)	4 vs 6	1 vs 9	0.3
Height (cm $\pm$ SD)	164.3 $\pm$ 12	161 $\pm$ 10.5	0.52
Weight (kg $\pm$ SD)	85.1 $\pm$ 22.1	73.2 $\pm$ 14.4	0.17
Body mass index (kg/m ² $\pm$ SD)	31.1 $\pm$ 5.1	28.6 $\pm$ 7	0.37
Race (White/other)	9 vs 1	9 vs 1	0.26
ASA class (II/III)	8 vs 2	7 vs 3	0.63
Baseline creatinine (mg/dl $\pm$ SD)	0.64 $\pm$ 0.3	0.71 $\pm$ 0.2	0.5

Results

Total tourniquet times were longer in the bilateral patients (44.5 ± 15.7 min for unilateral TKA patients and 101.5 ± 28.5 min for bilateral TKA patients, $p < 0.001$). Intraoperative estimated blood loss was less for unilateral patients (220 ± 42 ml for unilateral TKA patients and 385 ± 113 ml for bilateral TKA patients, $p = 0.001$). Bilateral patients required more units of transfused blood (0.7 ± 0.8 units of blood for unilateral TKA patients and 2.3 ± 1.4 units of blood for bilateral TKA patients, $p = 0.006$).

Baseline desmosine to creatinine ratios were higher in the unilateral as compared to the bilateral TKA group ($p = 0.003$).

Overall, desmosine levels increased in both groups, but the rise was significant only in the bilateral TKA group (Fig. 1a, b). The regression analysis of data from the bilateral TKA group showed no significant difference between baseline and postoperative day 1 ($3 \pm 6\%$ increase, $p = 0.517$). However, the desmosine level was found to be significantly higher at postoperative day 3 compared to baseline values ($62 \pm 18\%$ increase, $p < 0.001$) and postoperative day 1 ($56 \pm 20\%$ increase, $p = 0.001$). For unilateral TKA patients, the increase in urinary excretion of desmosine in the postoperative period was not different from preoperative levels (baseline vs. postoperative day 1 ($2 \pm 12\%$ increase, $p = 0.876$); baseline vs. postoperative day 3

($32 \pm 21\%$ increase, $p = 0.14$); postoperative days 1 vs. 3 ($29 \pm 26\%$ increase, $p = 0.2$; Fig. 2)).

Of note, the patient with the largest increase in desmosine levels (54.2 pmol desmosine per milligram creatinine at baseline to 176.1 pmol desmosine per milligram creatinine on postoperative day 3) received a unilateral procedure but had an extensive history of pulmonary disease including bronchiectasis and tuberculosis. However, no patient in this study developed clinically significant pulmonary complications.

Discussion

In this pilot study, we were able to identify an increase in urine desmosine levels after TKA surgery. However, this increase was significant only in the patient group undergoing bilateral procedures.

Our study is limited by a number of factors. First and foremost, it has to be acknowledged that this is a pilot study with limited patient numbers. Thus, the fact that the increase in desmosine levels in the unilateral group did not reach significance may be secondary to insufficient power.

Further, although we collected data on unilateral and bilateral TKAs, this investigation was not designed to be a

Fig. 1. **a** Urine desmosine/creatinine levels at baseline and 1 and 3 days after bilateral total knee arthroplasty (mean $\pm$ standard deviation). Significant differences of levels were found between baseline and day 3 and day 1 and day 3. **b** Urine desmosine/creatinine levels at baseline and 1 and 3 days after unilateral total knee arthroplasty (mean $\pm$ standard deviation). No significant differences were found between levels at different time points

Fig. 2. Percent change of urine desmosine/creatinine levels compared to baseline values. Only the rise of levels on day 3 for bilateral total knee arthroplasty (BTKA) patients was statistically significant (see *star*)

true case control study. Unilateral versus bilateral procedures were included in order to establish a measure of dose response, rather than perform a randomized study comparing the two procedures. By including all comers, we collected data on a cross section of two different patient populations that tend to undergo these two procedure types. Bilateral TKA recipients tend to be younger and may therefore have a lesser degree of age-related decrease in pulmonary capacity. This may explain the lower baseline desmosine levels in the latter patient group. However, it was beyond the scope of this study to correlate baseline levels of desmosine to preexisting levels of lung disease. Further, the small number of patients in this study may have limited our ability to show statistical differences in the demographic characteristics of these patient groups, although our clinical experience and the literature suggest that they do exist [17].

An additional limitation is linked to the inability to differentiate desmosine from various sources in the body (even if their contribution in this acute setting may at least in theory not be large). Although elastin is not exclusive to pulmonary tissue, the extracellular matrix of the lung is a major source of this protein and in the absence of injury elastin is stable over a person's lifetime [4, 9, 10, 13, 15]. Although a large portion of the elastin in the human body is found in the lung, the contribution of other sources, such as ligaments injured during surgery, is unknown. With current methods, it is not possible to distinguish desmosine origination from various body sources. Despite this limitation, there are a number of points supporting the utility of urine desmosine as a marker of lung injury during knee surgery. For example, the anterior and posterior cruciate ligaments, the most likely source of extrapulmonary elastin during knee surgery, were routinely removed, thus eliminating these structure as a major source of desmosine. However, elastin may have been released during retraction of the collateral ligaments during surgery. The quantification of the contribution of desmosine from the previously mentioned nonpulmonary sources will have to be the focus of future studies.

Therefore, results of this study would suggest that the measurement of changes in urine desmosine levels may be most useful on a qualitative basis in comparative studies targeted to evaluate specific lung protective interventions, thus largely negating the aforementioned limitation.

Overall, desmosine levels increased in both groups, but the rise was significant only in the bilateral TKA group. It is likely that the degree of lung injury is directly related to the total load of embolic material entering the pulmonary circulation. The higher incidence of pulmonary complications that we have reported in epidemiologic studies among bilateral TKA patients compared to those having unilateral procedures could be explained by this hypothesis [17]. In an analysis of nationally representative data, we found an incidence of 1.1% during UTKA and 1.9% during BTKA in pulmonary complications and a similar discrepancy in the incidence of ARDS [17]. Restrepo et al. [18] similarly found a doubling in the odds of pulmonary complications with BTKA versus UTKA. The results of our study provide further support for this theory and may explain the approximately twofold increase of urine desmosine in the bilateral (62%) versus the unilateral TKA group (32%) when compared to baseline (preoperative) values. Additional insults that may contribute to the higher incidence of lung injury seen among bilateral TKA patients include blood product transfusion-related factors [19], which were also more frequent in the bilateral group in our study.

We found a peak in urine desmosine levels on the third postoperative day. Animal studies have shown that after rapid release into the blood stream desmosine sequesters in the kidney tissue followed by slow release into the urine [15]. These kinetic data may in part explain the peak in desmosine levels on day 3 in our study. Further, this finding may mirror delayed breakdown of elastin after the initial insult to the lung and/or additional postoperative insults that lead to a protracted inflammatory response. Similar kinetics were found in a previous study of ARDS patients [12].

Further insight into the value of desmosine as a marker of pulmonary injury in our study may be provided by the observation that the patient with the highest urine desmosine to creatinine ratio after surgery had a history significant for preexisting pulmonary disease in the form of bronchiectasis and tuberculosis, suggesting increased vulnerability to secondary insults.

In summary, we were able to identify a significant increase in urine desmosine levels associated with bilateral but not unilateral knee surgery. In the context of previous studies, our findings suggest that desmosine may be a marker of lung injury in this setting. However, further research is warranted for validation and correlation of desmosine levels to clinical markers and various degrees of lung injury.

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