Risk of *S. aureus* infection may be greater day 1 post-op due to blood loss and saline dilution of natural humoral immunity which recovers within 2-weeks: A natural history study of healthy total knee arthroplasty patients

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INTRODUCTION: Total knee arthroplasty (TKA) is one of the most frequently performed surgeries annually in the US typically resulting in successful outcomes which include reduced pain and improved function. Patient optimization, and standardized infection prevention protocols in surgery and in the postoperative period have greatly helped reduce the risk of surgical site infection (SSI).[1-3] Still, around 0.5.-1% of patients develop postoperative periprosthetic joint infection (PJI) most often caused by *Staphylococcus aureus*.[4] This remains a serious risk even though the incidence of PJI is very low after elective primary TKA. Our research regarding this has focused on the immune proteome against *S. aureus* with the theory that an independent variable of a host's response to *S. aureus* is its aggregate humoral immunity comprised of all circulating antibodies against *S. aureus* antigens known as the "immune proteome".[5] Of note, individual antibodies can be irrelevant (non-neutralizing, non-functional, and/or against antigens that do not influence the outcome[6]), protective (e.g. anti- autolysin (Atl) that neutralize vital proteins, are opsonophagocytic and/or immunomodulatory)[7], or pathogenic (e.g. anti-iron surface determinant (Isd) that exacerbate infection and/or sepsis[8-10]). To identify unknown risk factors, we completed a natural history study of IgG specific for *S. aureus* antigens previously phenotyped as protective (anti-Atl) and pathogenic (anti-Isd).

METHODS: 25 male and 25 female patients 50-85 years of age and BMI 24-39 undergoing primary TKA were prospectively enrolled into a natural history study. Patients underwent rigorous pre-operative optimization, and serum levels of anti-staphylococcal antibodies and anti-tetanus toxoid IgG levels (unrelated to *S. aureus*) were determined via custom Luminex assays and were compared to clinical blood studies. Using anti-Gmd IgG (i.e., anti-Atl) serum concentrations determined via Luminex assay,[21] and an established clinically relevant threshold for osteomyelitis patients of 100ug/ml,[22] a sub-analysis was performed of the change in hemoglobin (Hgb) and anti-Gmd IgG concentration from pre-op to post-op Day 1. Clinical, demographic, KOOS and PROMIS-10 data were also collected with outcomes to 2-years post-op.

RESULTS: All participants completed the study and 2-year follow up. Reported outcomes were excellent with none of the patients readmitted nor developing a surgical site infection or serious adverse event. Serology revealed a highly significant decrease in 7 out of 9 antibody titers on Day 1 ($p<0.0001$), with all but 1 returning to preoperative levels within 2 weeks (Table). These antibody decreases coincided with a 20% drop in hemoglobin 13.8 +/- 1.7 at preop to 11.1 +/- 1.8 mg/dL on Day 1 (Figure). Protective anti-Gmd IgG concentrations for the 20 patients whose pre-op level was >20ug/ml dropped in similar fashion from pre-op to Day 1 post-op (Figure). None of these 20 patients experienced a decrease below 10ug/ml at post-op Day 1. Of note, the patients with the two highest anti-Gmd titers were recovering from UTI, and the patient with the 3rd highest titer was recovering from prostatitis prior to surgery but all were resolved by the time of surgery (Figure). Cytokine data (not shown) was mostly unremarkable though, at Day 1, significant elevations were seen in CRP and IL-6 while significant reductions were seen in IL-17F, TNF-β, and IL-28A. Levels of IL-6, IL-17F, TNF-β and IL-28A returned to near baseline by 2 weeks after surgery. CRP levels remained elevated at 2 weeks and returned to near baseline by 6 weeks.

DISCUSSION: Infection is a major concern after TKA and one of the most common causes for failure of an otherwise well-done TKA.[13] *S. aureus* remains the most common etiology for PJI following TKA.[14] Our observed drop in anti-*S. aureus* antibodies corresponded with blood loss. Though this immune deficiency recovered by 2-weeks post-op and had no clinically significant effects it occurs at the most vulnerable time while the patient is actively working to heal the surgical wound and prevent implant colonization from bacteria that may have entered the surgical site.

SIGNIFICANCE/CLINICAL RELEVANCE: Optimized patients undergoing elective TKA experience significant reductions in humoral immunity coincident with blood loss and hemodilution. This dynamic change in anti-staphylococcal antibodies may cause an imbalance between protective and pathogenic antibodies which may increase the risk of PJI for some patients.

REFERENCES: 1) Johns WL, et al. Tot Joint Arth. (2020); **2)** Childers CP, et al. Ger. Orth Surg Rehab. (2018); **3)** Van Horne A and Van Horne J. Arth Today. (2020); **4)** Wang JC, et al. JBJS Am. (2022); **5)** Master EA, Bone Res. (2019); **6)** Owen JR, et al. Eur Cell Mater. (2021); **7)** Varrone JJ, et al. J Orth Res. (2014); **8)** Nishitani K, et al. JCI Insight. (2020); **9)** Muthukrishnan G, et al. J Orth Res. (2021); **10)** Muthukrishnan G, Curr Osteoporosis Rep. (2019); **11)** Lee et al. Eur Cell Mater. (2020); **12)** Kates SL, et al. JBJS. (2020); **13)** Lum ZC, et al. World J Orth. (2018); **14)** Tai DBG, et al. Clin Micro Inf. (2022)

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Table: TKA patients experience a decrease in anti-S. aureus humoral immunity post-op Day 1 that recovers by 2-weeks. Sera from all 50 TKA patients obtained at the indicated time points were analyzed for IgG specific antibodies for the indicated antigens via custom Luminex assay and the titers are presented below as the mean of median fluorescent intensity (MFI) +/- SEM (statistical differences vs. pre-op are shown as *p<0.0001 and ● p<0.05).

Time point	IsdA (MFI)	IsdB (MFI)	IsdH (MFI)	Amd (MFI)	Gmd (MFI)	CHIPS (MFI)	SCIN (MFI)	Hla (MFI)	TetTox (MFI)
Preop n=50	3043 *(491)*	7477 *(886)*	1350 *(181)*	4864 *(455)*	2509 *(286)*	3567 *(608)*	2778 *(409)*	2392 *(319)*	3475 *(442)*
Postop Day 1 n=50	2360* *(375)*	6419* *(759)*	1179 *(144)*	3863* *(369)*	2030* *(236)*	2931* *(538)*	2247* *(333)*	1969 *(277)*	2740* *(365)*
2 weeks n=46	2890 *(494)*	8063 *(983)*	1714● *(237)*	4866 *(465)*	2400 *(272)*	3308 *(559)*	2705 *(441)*	2470 *(418)*	3550 *(464)*
6 weeks n=45	2975 *(483)*	8055 *(933)*	1668 *(203)*	5165 *(459)*	2700 *(316)*	3244 *(462)*	2818 *(437)*	2758 *(460)*	3281 *(374)*
12 weeks n=50	2997 *(463)*	7853 *(840)*	1645 *(266)*	5159 *(436)*	2658 *(284)*	3532 *(588)*	2861 *(401)*	2672 *(413)*	3388 *(419)*

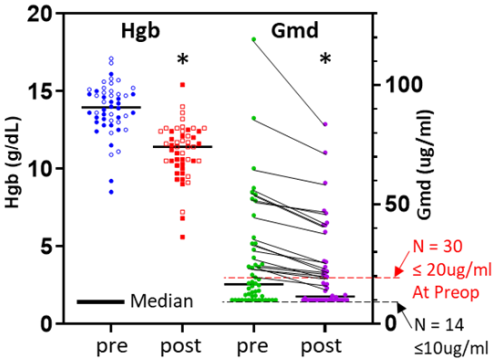

Figure: TKA patients experience a decrease in blood hemoglobin (Hgb) and anti-Gmd IgG antibody concentration at post-op Day 1. *p<0.05 vs. pre-op