**Introduction:**
The relationship between systemic glycocalyx degradation markers and regional glycocalyx thickness in non-septic critically ill patients is unclear. Conjunctival sidestream dark field-imaging for the purpose of glycocalyx thickness estimation has never been performed. We aimed to investigate whether changes in glycocalyx thickness in conjunctival and sublingual mucosa are associated with global glycocalyx shedding markers.

**Methods:**
In this single-centre prospective observational study, using techniques for direct in-vivo observation of the microcirculation, we performed a single measurement of glycocalyx thickness in both ocular conjunctiva and sublingual mucosa in mixed cardio surgical (n=18) and neurocritical patients (n=27) and compared these data with age-matched healthy controls (n=20). In addition we measured systemic syndecan-1 levels.

**Results:**
In the sublingual and conjunctival region we observed a significant increase of the perfused boundary region (PBR) in both neuro critical and cardiac surgical ICU patients, compared to controls (2.20[2.04-2.42] vs 1.76[1.63-2.08] and 2.19[2.01-2.36] vs. 1.70[1.61-2.00], p<0.05). There was a significant increase of syndecan-1 in ICU patients comparing with controls and in cardiac patients comparing with neurological (120.0[71.0-189.6] vs. 18.0 [7.2-40.7], p<0.05). We detected a weak correlation between syndecan-1 and sublingual PBR(r=0.40, p=0.002) but no correlations between global glycocalyx damage markers and conjunctival glycocalyx thickness.

**Conclusion:**
Conjunctival glycocalyx thickness evaluation using SDF videomicroscopy is suitable and is impaired in non-septic ICU patients but only measurements in sublingual mucosa are correlating with systemic glycocalyx shedding markers. Global glycocalyx damage is more severe in cardiac comparing to neuro critical patients.