Thrombus aspiration (TA) is a recommended technique in the treatment of myocardial infarction allowing thrombus removal from the culprit artery via a specific catheter. A reduced mortality in patients affected by myocardial infarction with ST elevation (STEMI) has been associated to the use of TA in conjunction with primary percutaneous intervention (PCI) (De Luca et al. 2008; Burzotta et al. 2009). Noteworthy, TA allows to collect the biological aspirate in a minimally invasive way for the characterization of its morphology and composition that can trace the evolution of the pathology from the lesion of the atherosclerotic plaque to the reperfusion of the ischemic cardiac tissue (Silvain et al. 2011). We aimed at developing and applying a multiscale approach to determine coronary thrombus composition by means of optical and electron microscopic techniques on a series of 57 thrombi collected during PCI with TA on STEMI patients.

Each thrombus was immediately fixed (2% glutaraldehyde in 0.1M phosphate buffer) and a digital colour image (10x) was obtained for the qualitative macroscopic classification into “red”, “mixed” and “white” thrombus (Figure 1). The sample was subsequently prepared and imaged by SEM in high vacuum mode (2000x) for percent quantification of platelets, red blood cells (RBCs), white blood cells (WBCs) and fibrin (Figure 2) according to a method we developed for thrombi in hemodialysis catheters (Lucas et al. 2013). Scanned samples were then rehydrated and processed for permanent histology. Platelet, RBCs and WBCs were quantified on Carstair’s stained sections by setting specific colour thresholds in the La*b* colour space (Figure 3).

Macroscopic classification according to two senior and a young cardiologists gave 8(14%) “white”, 20(35%) “mixed” and 29(51%) “red” thrombi. Inter-observer agreement was good for “red” and “white” thrombi, but lower for the identification of mixed thrombi. The mean (SD) histological composition was 18%(20%), 47%(24%) and 35%(20%) for platelet, RBCs and WBCs respectively. Features quantification on SEM images was feasible in 53/57 samples giving a mean (SD) SEM composition of 31%(25%), 40%(22%), 26%(19%), and 3%(3%) for platelets, RBCs, WBCs and fibrin respectively. Sub-groups analysis showed an agreement between macroscopic classification and both SEM and histological composition: white thrombi presented a mean prevalence of platelets (42% at histology and 58% at SEM) while red thrombi were mostly composed of RBCs (59% at histology and 42% at SEM). Intermediate features were found for mixed thrombi.

Method here presented deserves high potential for understanding the mechanisms of thrombus formation in STEMI and for investigating correlations between composition and thrombus age or drug treatments.

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Fig. 1: Macroscopic classification of aspirated coronary thrombi. Representative image of a white (a), mixed (b), and red (c) sample.

Fig. 2: SEM images of the thrombus surface. Representative fields of view with a prevalence of platelets (A), RBCs (B), WBCs (C, stars), and fibrin (D). High vacuum mode, 10 KV, SE detector, 2000x original magnification.

Fig. 3: RBCs, fibrin, and platelets on a mixed thrombus section stained with Carstair’s method (left). Representative colours associated to the three components of interest are shown. Binary images after specific threshold for RBCs (a), fibrin (b), and platelets (c). Inset d) reports about components not recognized in the three previous features.