recognised: Signal 1 or priming (expression of NLRP3 protein, pro-IL-1β, pro-IL-18 and pro-Caspase-1), followed by Signal 2 or activation (active IL-1β and IL-18). Additionally, Caspase 5 may be involved via a non-canonical pathway. The purpose of this study was to investigate the possible role of mitochondrial and cytosolic superoxide in activating the NLRP3 inflammasome in THP-1 and HUVEC cells. THP-1 cells were differentiated with PMA (50ng/ml) for 1-3 days. Subsequently, LPS treatment (0.1µg/ml) was administered for 24 h for Signal 1 activation followed by ATP (500µM) for 1 hour for Signal 2. The effects of intracellular generation of superoxide (mitoparapet and paraparquat at 1 and 5µM) was investigated before and after LPS, NLRP3, pro-IL-1β, pro-IL-18, pro-Caspase-1 and -5 were detected in THP-1 cell lysates by Western blotting and active IL-1β and IL-18 detected in cell culture supernatants by ELISA. In our hands, intracellular superoxide generation did not activate the NLRP3 inflammasome in PMA-differentiated THP-1 cells nor did it inhibit LPS-induced priming. Both endothelial cell types showed evidence of low level Signal 1 activation. Despite HUVEC cells showing priming of the NLRP3 inflammasome, there was no evidence for either active IL-1β or active IL-18 synthesis. Surprisingly, EA.hy926 cells showed low levels of active IL-1β, when exposed to PMA, suggesting other end-points of an active NLRP3 inflammasome are worthy of investigation.

P152
The Q222R deoxyribonuclease I single nucleotide polymorphism is associated with mortality in patients after ST-elevation myocardial infarction

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Background: Neutrophils are able to release their nuclear content into extracellular space by formation of neutrophil extracellular traps (NETs). NETs are capable to neutralize pathogens, but have also been implicated in autoimmune and thrombotic diseases, including ST-elevation myocardial infarction (STEMI). Deoxyribonuclease (DNase) I degrades NETs. DNase I Q222R single nucleotide polymorphism (SNP), which impairs DNase I function, was associated with an increased incidence of MI. In STEMI, impaired DNase I activity was correlated with increased NET burden and infarct size. In a mouse model of coronary artery ligation, DNase I treatment decreased infarct size, indicating a potential therapeutic role.

Purpose: We hypothesized that DNase I is crucial to counteract dysregulated NET formation in coronary artery disease (CAD). The Q222R SNP in the DNase I gene, resulting in dysfunction of the enzyme, might thereby induce chronic NET burden with influence on long-term outcome.

Methods: We enrolled CAD patients with a history of STEMI which received primary percutaneous coronary angioplasty between 2012-2016 (n = 211). Genotyping using allelic discrimination qPCR was performed to identify DNase I Q222R (rs1053874). Mortality data was obtained from the national registry of death. Causes of death were classified according to ICD-10. By multivariable Cox regression, we assessed the influence of DNase I SNP on all-cause and cardiovascular mortality, adjusting for the following established cardiovascular risk factors: age, sex, body mass index, diabetes, smoking, hyperlipidemia, renal function as measured by serum creatinine concentration at admission and arterial hypertension.

Results: Homozygous mutation of the DNase I SNP was present in 44 (9.0%) patients; 304 (42.8%) and 341 (48.2%) were heterozygous and homozygous for the wild-type allele, respectively. Median survival was 600 [interquartile range 30.3, 91.5] months. A total of 133 (18.7%) patients deceased; 78 (11.0%) died of cardiovascular causes. Homozygous mutation of DNase I was independently associated with all-cause mortality (hazard ratio 2.05, 95% CI 1.22-3.46, p = 0.006) and cardiovascular mortality (hazard ratio 2.02, 95% CI 1.02-4.01, p = 0.046).

Conclusion: We report a negative influence of the Q222R DNase I SNP on survival after STEMI. Our findings argue for a deleterious role of NETs only not in CAD.