ular (LV) remodelling after acute MI, but the mechanism of this improvement has never been assessed. We evaluated the relationship between ECFC levels and microvascular obstruction (MVO), and the impact of this relation on infarct size and LV remodelling at 6 months as assessed by magnetic resonance imaging (MRI).

**Methods:** 109 pts≥75 years old, admitted with a first MI within 12 hours of onset of symptoms were enrolled. Peripheral blood samples were drawn to assess number of ECFC colonies (culture measurements). Measurements of infarct size by MRI were performed at day 5 and 6 months.

**Results:** ECFC colonies were detected in 51/109 pts (47.2%) at admission (ECFC<sup>pos</sup> pts). At 5 days, MVO was more frequently observed (63% vs 33%; p=0.003) and of greater magnitude (7±6% vs 3±5%, p=0.0004) in ECFC<sup>pos</sup> patients versus ECFC<sup>neg</sup> pts respectively. At 6 months, there was a significantly greater reduction in infarct size in ECFC<sup>pos</sup> pts (–33.7±33.2% vs –15.1±24.6%, ECFC<sup>pos</sup> vs ECFC<sup>neg</sup> respectively; p=0.003). This reduction in infarct size was associated with a significant improvement in LV ejection fraction and a significant reduction in LV end diastolic and systolic volumes in ECFC<sup>pos</sup> pts. A significant positive correlation was observed among ECFC<sup>pos</sup> pts between MVO at day 5 and infarct size at 6 months (r=0.58, p<0.001), while the number of ECFC colonies was significantly correlated with the relative change in infarct size at 6 months MRI (r=0.33, p=0.0001).

**Conclusion:** The presence of ECFC colonies is associated with a reduced degree of microvascular obstruction early after myocardial infarction, leading to reduced infarct size and positive LV remodelling at 6 months and can be considered as a marker of microvascular integrity in acute MI pts.

**Validation of assessment of circulate oxidative stress markers by the Free Oxygen Radicals Testing (FORT) assay among patients with an acute myocardial infarction.**

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**Background:** Free oxygen radicals play an important role in the pathogenesis of many diseases including cardiovascular diseases, diabetes, cancer and aging. Several methods were developed for the direct or indirect measurement of oxygen free radical and its by-products. Using a new Free Oxygen Radicals Testing (FORT) the current study is designed first to validate the device and to investigate the potential relationships between the ROS and clinical or biological factors in human serum from a population of men with an acute myocardial infarction (AMI).

**Methods:** We first determined the effect of storage, variability and reproducibility of the FORT test in serum. Then we used the test in 66 patients from our bio bank of AMI patients.

**Results:** FORT values vary between 324 and 1198 FORT units, with a median value of 581 (494-754) FORT units. Among the risk factors, 17% of patients are diabetic, and 20% are obese. In univariate analysis, the FORT values seem to be influenced by age (r=0.161, p=0.195), presence of diabetes (p=0.102), a history of MI (p=0.181), LVEF <40% (p=0.005) and treatment with blockers before admission (p=0.053), with ST-Elevation MI (p=0.058), levels of CRP (r=0.438, p<0.001), the rate of neutrophil (r=0.203, p=0.107) and peak CK (r=0.274, p=0.028). The analysis of multiple linear regression showed that CRP (p=0.023), LVEF <40% (p=0.001) and presence of diabetes (p=0.039) were independent predictors of serum FORT levels. This statistical model can explain 45% of the variance in the FORT levels.

**Conclusions:** The variability of the FORT on serum is minimal and thus reproducibility can be attained. FORT assay is stable when stored at 20 °C for a couple of months or at 4 °C for a few days. FORT correlation with CRP, LVEF and status of diabetes provides an interesting insight and a good link between oxidative stress and inflammation in patients with an AMI.

**The polymorphism Trp719Arg in the kinesin-like protein 6 is associated with the presence of late outgrowth endothelial progenitor cells in acute myocardial infarction**

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**Background:** Much attention is focused on genetic polymorphisms associated with coronary artery disease. A candidate gene controversially associated with risk of coronary events is the kinesin-like protein 6 (KIF6), which is correlated with Trp719Arg polymorphism. In acute myocardial infarction (AMI), endothelial progenitor cells, particularly endothelial colony forming cells (ECFC) are mobilized from bone marrow and correlated with infant size reduction. We investigated whether there was a relationship between presence of ECFC in AMI at admission and genetic status with regard to the KIF6 Trp719Arg polymorphism (rs20455).

**Methods:** Forty five patients aged ≤75 years old referred for a first STEMI or non STEMI AMI. Peripheral blood samples were drawn on admission. Isolated peripheral blood mononuclear cells were obtained by Hypaque-Ficoll density gradient centrifugation and cultured for 4 weeks. Cultured cells were phenotyped to assess the endothelial origin of ECFC. Genomic DNA was extracted in all patients and genotyping for allelic variations of KIF6 was performed.

**Results:** Subjects were divided into two groups comparing the (Arg/Arg) homozygote variants with patients having a Trp allele. The genotype frequencies were 55%, 31% and 13% for Arg/Arg, Arg/Trp and Trp/Trp respectively. Between groups, higher levels of Trp, CK and CK-MB were observed in the Arg/Arg group (respectively p=0.026, p=0.001 and p=0.031). ECFC were observed in 33% of patients with AMI. The percentage of patients without ECFC was significantly higher in the Arg/Arg group (p=0.033). No other significant differences were observed between groups.

**Conclusion:** In this report the Arg/Arg group showed a high number of ECFC-negative patients. A possible explanation might be the low mobilization of ECFC from bone marrow in this genotype since KIF6 is involved in cytokinesis. The altered amino acid amino acid Trp719Arg could decrease the ECFC released in AMI at admission and genetic status with regard to the KIF6 Arg/Trp polymorphism (rs20455).

**Renin-angiotensin-aldosterone system polymorphisms : a role or a hole in occurrence and long-term prognosis of acute myocardial infarction at Tunisian older people**

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**Background:** Myocardial infarction (MI) is one of the major causes of death all over the world. Because MI frequently occurs suddenly without any preceding clinical symptoms, the prediction of MI is clinically of great importance. Angiotensin II is produced primarily by angiotensin I-converting enzyme (ACE) within atherosclerotic lesions and ACE level correlates with the severity of vessel wall damage. We analyzed the evolution with age of the frequencies of the ID polymorphism of the ACE, A1166C of the angiotensin II AT1 receptor (AT1R), and M235T of the angiotensinogen (AGT) gene in a healthy population and he subsequent comparison to age- and sex-matched groups of MI patients.

**Aim:** To investigate the influence of increasing age on the incidence and remaining lifetime risk of myocardial infarction in a cohort of older men.

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Methods: Polymorphisms of the AGT, ACE (I/D) and AGTR1 (A1166C) genes in patients and controls were analyzed using allele-specific PCR, and PCR-rflp.

Results: A total of 140 healthy subjects and 119 individuals with MI were divided into two groups < 65 and > 65 years old. The evolution with age showed that the AGT M allele (P < 0.001) and the ACE I allele (P < 0.05) frequency decreased with age. The TT genotype frequency increased in patients with MI > 65 years (OR = 3.52, 95% CI: 3.12-5.54). The AA genotype showed a similar behaviour (OR = 2.9, 95% CI: 1.1-3.6). The DD genotype increased in the MI > 65 group (OR = 6.66; 95% CI: 2.02-21.9). Serum ACE activity was positively associated to age (r=0.38, p=0.000). Patients with DD genotype > 65 years was significantly higher (119.7 ± 50.8 U/L) than in patients with DD genotype < 65 years (96.9 ± 34.1 U/L, p=0.039).

Conclusions: The incidence of new cardiovascular disease continued to increase after age 65 but was most often diagnosed at death. These findings suggest that people aged 60 and older have a substantial amount of undiagnosed disease. We propose that the study of the allele frequency evolution in an healthy population at different ages is essential to determine risk factors for MI in case-control studies.

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Plakophilin2b is not expressed in the heart : consequences for the genetic screening of arrhythmogenic right ventricular cardiomyopathy

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Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart disease caused predominantly by heterozygous Plakophilin-2 (PKP2) mutations. However, few molecular studies have been performed to ascertain their pathogenicity. PKP2 encodes two isoforms, the longest (PKP2b) including the alternatively spliced exon 6, which is routinely screened for molecular diagnosis, despite the absence of data on the cardiac expression of PKP2 isoforms. This study addressed the pathogenicity of exon 6 mutations by focusing on a heterozygous missense p.Arg490Trp variant in PKP2.

Methods and results: The p.Arg490Trp variant of PKP2 was identified in two unrelated ARVC probands and absent from 470 controls. In silico analysis suggested that PKP2 exon 6 is an Alu-derived sequence with very low expression level. Transcriptional and Western blot analysis confirmed that the exon 6 missing in PKP2a was the only clearly detectable isoform in all heart samples (four controls and a proband). Moreover, in the proband’s heart sample, the variant was not associated with aberrant exon 6 splicing or mutant mRNA down-regulation. Finally, we identified in this proband a heterozygous missense variant (p.Gln2343Lys) in the desmoplakin gene likely to be the disease-causing mutation.

Conclusion: We demonstrated that only PKP2a is significantly expressed in the heart. Our results strongly suggest that the p.Arg490Trp mutation and other variants located in PKP2 exon 6 can not be considered as disease-causing mutations and therefore that PKP2 exon 6 screening is useless in routine ARVC molecular diagnosis. These results have important consequences for the interpretation of PKP2 mutation screening and subsequent genetic counseling.

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Effects of the selective heart rate-reducing agent ivabradine on the risk of onset of ventricular fibrillation during myocardial ischemia in pigs : involved mechanisms.

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Aim: A major risk of myocardial ischemia (MI) is the onset of ventricular fibrillation (VF). Our study evaluated the effect of heart rate reduction (HRR) induced by ivabradine (IVA), the first selective inhibitor of the pacemaker current If, on the risk of onset of VF, and study the possible mechanisms involved.

Methods: MI was induced in pigs by total occlusion of the anterior interventricular artery until onset of VF, which was assessed by the VF threshold (VFT; protocol 1) or the time to onset of VF (TOF; protocol 2), with or without IVA (0.25mg/kg, i.v.). Electrocardiographic, hemodynamic parameters and ischemic area were assessed in these two protocols. The impact of IVA was evaluated on: i) ultrastructure of mitochondria (by electron microscopy) in protocol 1; ii) myocardial energetic status compared with a β-blocker, propranolol (PROP) in protocol 2; iii) regional myocar-dial blood flow (RMBF) after 1 min-ischemia followed (without VF) by reperfusion by positron emission tomography in protocol 3.

Results: IVA induced a significant HRR (~25%), increased the VFT and the TOF. When compared to control, IVA prevented the apparition of myocardial reperfusion-induced VF and maintained myocardial energetic status. Although further work is needed to determine the mechanisms underlying these effects, this might give a place of choice to IVA in the prevention of ischemia-induced VF.

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The spontaneously hypertensive rat heart displays a reduced capacity to use long chain fatty acids for energy production prior to hypertrophy : role of Cd36.

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Aim: The spontaneously hypertensive rat heart is a model of hypertension with a Cd36 gene defect, which leads to left ventricular hypertrophy (LVH) between 9 and 12 weeks. At 15 weeks, perfused SHR hearts display a reduced capacity to oxidize long chain fatty acids (LCFAs) but not carbohydrates (CHO) for energy production. In this study, we have further examined LCFA metabolism and its potential dysregulation in SHR hearts prior to LVH.

Methods: SHR and control Wistar rat hearts (7 weeks) have been used for i) functional and metabolic phenotyping using ex vivo perfusion with [14C]-labeled substrates, ii) metabolic gene expression profiling using qPCR, iii) studies of mechanisms regulating -oxidation (malonyl-CoA, AMPK) and of the functional impact of the gene mutation in the LCFAs transporter Cd36 (western blot, confocal microscopy, qPCR, gene analysis).

Results: Compared to controls, 7-week-old SHR hearts display a lower contribution of LCFA (i.e. oleate) to oxidation (40%) and triglyceride (2.8-fold) formation, which is associated with a compensatory increase in CHO oxidation. These alterations cannot be explained by changes in metabolic gene expression or common mechanisms regulating -oxidation. Western analyses demonstrate that compared to controls, cardiac Cd36 protein level is lower in the SHR, while data from confocal microscopy indicate a similar intracellular localization. Sequence alignment of Cd36 cDNA indicates non-synonymous...