followed by reoxigenation (8-24-30-48 h) and after exposure to C-reactive Protein (CRP: 1-5-10-20-50 μg/ml), Angiotensin II (Ang II: 1-2.5-5 μM), IL-6 (1-10-100 ng/ml), TNF-alpha (1-5-10 ng/ml), Acetylcholine (Ach) and Epinephrine (Epi) (1-10-100 μM) for 24 and 48 h.
**Results.** After 16 h of hypoxia there was a significant reduction in total Cx43 protein by 50%. After 24 and 48 h of hypoxia Cx43 protein decreased by 75% and 90%, respectively (P<0.01). Cx40 protein level was unaltered after hypoxia treatment. A progressive reduction in Cx43 IF occurred at site of intercellular junctions. After 24 h of hypoxia, Cx43 mRNA levels decreased by 35% and at 48 hours by 42% (P= 0.007). Similar results were obtained by SI in normoxic condition. The association of hypoxia and SI did not result in a decrease of Cx43 protein levels. After 24 h of hypoxia, Cx43 protein levels increased up to basal level within 48h of reoxygenation. This normalization of Cx43 protein levels with reoxygenation was not observed after 48 h of hypoxia. CRP, IL-6, Ang II, TNF-alpha, Ach, Epi, didn't change Cx43 and Cx40 expression.
**Conclusions.** Hypoxia or SI downregulate Cx43 protein expression in atrial cardiomyocytes. Hypoxia-induced Cx43 downregulation is reversible depending on hypoxia duration. Of note, the association of hypoxia and SI did not change Cx43 levels. These alterations might contribute to the generation of an atrial arrhythmogenic substrate.

## C339

**3-D FIBRIN SCAFFOLD IMPROVES STEMNESS OF HUMAN ENDOTHELIAL PROGENITOR CELLS**

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**Aims.** Fibrin is a natural biopolymer appealing for cell-based regenerative therapies, because it can support growth, migration and differentiation of different cell types. Endothelial progenitor cells (EPC) represent a very interesting alternative cell source for mature endothelial cells; the fact that can easily isolated from the peripheral blood, thereby eliminating donor morbidity, makes them ideal in applications in the field of regenerative medicine. We have demonstrated that fibrin can support EPC viability and growth.
Aim of this study was to evaluate if fibrin can affect EPC differentiation and stem cell markers expression.
**Methods.** Fibrin was prepared mixing commercially available (Kedrion S.p.A. Lucca, Italy) fibrinogen (9 mg/ml) and thrombin (25 U/ml). Clot ultrastructure was investigated by scanning electron microscopy (SEM) and cryogenic SEM (CRYO-SEM) to measure fibre diameter and density. Clot elasticity was evaluated by atomic force microscopy (AFM), measuring the tip-sample force by cantilever displacement. EPC were obtained from peripheral blood and cultured on fibrin at the concentration of $1x10^6$cell/cm$^2$. Fibronectin coating was used as a control. Metabolic activity was assessed after 7 and 14 days by WST1 assay and viability by confocal microscopy (calcein incorporation). The expression of both endothelial (CD31, KDR, vWF, Ve-Cadherin) and stem cell markers (nanog, oct-4) was assessed by flow cytometry, confocal microscopy and Real Time RT-PCR.
**Results.** SEM analysis revealed a nanometric fibrous structure, with mean fiber diameter of 165±4 nm and mean density of 95.9±0.2 %. CRYO-SEM suggested a reticulate structure with mesh-size up to 10 μm. Fibrin clot elasticity was 1.78 MPa, as in literature. WST1 assay showed that fibrin increased EPC metabolic activity as compared to fibronectin (fibrin: 0.606±0.056 a.u. vs. fibronectin: 0.311±0.067). Calcein staining demonstrated that EPC were still viable at 14 days. Flow cytometry showed the expression of endothelial markers (CD31=41.8±8.4%; vWF=32.3±3.0%; KDR=89.3±3.7%; VE-Cadherin=41.2±3.8%), confirmed also by confocal microscopy and Real Time RT-PCR. Interestingly, nanog and oct-4 (embryonic stem cell markers) expression was significantly greater on fibrin (p<0.001) as compared to fibronectin.
**Conclusions.** These findings suggest that fibrin it is not only a suitable scaffold for EPC growth and viability but also induces EPC differentiation. The observation that Nanog, known as the most important marker of stemness, is maintained longer than on fibronectin, may offer a surplus value to stem cell-based therapies.

## C340

**ASSOCIATION OF rs2200733 AT 4q25 WITH ATRIAL FLUTTER/FIBRILLATION DISEASES IN ITALIAN POPULATION**

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Atrial fibrillation (AF) and atrial flutter (AFL) are common cardiac conduction disorders affecting many individuals. Recent studies on sporadic cases of AF/AFL revealed a significant association of the single nucleotide polymorphism (SNP), rs2200733T, on chromosome 4q25 with these diseases, suggesting the critical function of the T-risk allele in disease development.
The aim of our study was to determine the association of rs2200733 in Italian patients with AF (n=45) or AFL (n=33).
We genotyped the SNP rs2200733 using a Taqman Assay in all patients with AF or AFL and in 348 Italian controls.
The case-control analysis performed by gPlink revealed that AF/AFL were strongly associated with the rs2200733 SNP. The risk-allele T of rs2200733 has an allelic frequency of 0.27 in AF and AFL patients combined and 0.15 in the controls.
Our results indicate that there is a positive, significant association between the rs2200733 T allele and Italian AF and AFL patients (p<0.0001 and p<0.00007, respectively). This is in agreement with a previously reported association study conducted on an Icelandic population, where the minor allele rs2200733T was found associated with AF and AFL.

### Interventistica coronarica

## C341

**CARDIO REGISTRY: SAFETY AND EFFICACY OF PACLITAXEL-ELUTING STENT UTILIZATION IN THE "REAL WORLD". TWO-YEAR CLINICAL FOLLOW-UP**

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**Introduction.** Early results with Drug-Eluting Stents (DES) have shown significant improvements of clinical and angiographic outcome in terms of restenosis. However, there are concernes about the very long- term efficacy (late restenosis) and safety (late thrombosis) of these stents beyond one year after implantation.
**Aim.** The purpose of this "real world" registry is to assess the very long-term safety and efficacy of Paclitaxel-Eluting Stents (PES) two years after implantation in an unselected population.
**Methods.** 504 consecutive pts underwent PCI with ≥1 PES implantation were enrolled in our single centre Registry and pts who received ≥1 PES in ≥1 vessel were included in the present analysis. Clinical and angiographic follow-up was conducted at 9 months after the procedure.At 24-month a clinical follow-up was performed by a telephone interview to ascertain whether they had experienced any MACE since 9-month examination.
**Results.** 504 consecutive pts (mean age 60.3±10.8, men 79.9%) underwent PCI with ≥1 PES implantation. Diabetes mellitus was present in 28.8%, in 7.8% requiring insulin therapy. A total of 561 PES were implanted with 1.6±0.6 PES/pt. A total of 484 vessels (1.4±0.6 vessels/pt) were treated with 1.4±0.7 PES/vessel. Pre- and post- procedural angiographic characteristics were: reference vessel diameter (RDV) 2.9±0.4mm, minimal lumen diameter (MLD) 0.7±0.5mm, lesion length 15.3±10.2mm, diameter stenosis 76.4±17.6%. The final MLD was 3.0±0.4mm. The 9-month follow-up (FU) was available in 95.7% of pts, with an angiographic FU performed in 69.5% of pts. Overall MACEs rate was 13.0%, cardiac death 2.1%, Q MI 3.3%, non-Q MI 0.9%, TVR 10.6%. Angio f-u showed: all re-PCI TLR 6.9%, late luminal loss 0.5±0.8mm.and binary restenosis rate 10.8%. No definite late stent thrombosis (ARC definition) occurred. Two-year clinical FU was performed in 86.8%. Overall MACEs rate was 16.9% (Death 3.8%, Q MI 3.7%, TVR 11.4%, TLR 7.3%). Non-cardiac death rate was 2.2% due to senectus (1 patient), stroke (1 patient), acute respiratory distress (2 pts) and suicide (1 patient). Late probable DES thrombosis rate was 1.3% occurred respectively 23,19 and 15 months after PES implantation and possible DES thrombosis rate was 0.4% occurred 15 months after procedure.LAST occurred in 1 pt at 4 years (0.2%).
**Conclusions.** The data regarding our experience about PES utilization in a "real world" population demonstrate sustained efficacy and safety up to two year after implantation, characterized by significant benefit in low rate of repeating revascularization and a very low risk of late stent thrombosis.

## C342

**IMPACT OF STRESS TESTING PRIOR TO PCI OR MEDICAL MANAGEMENT ON OUTCOMES OF PATIENTS WITH PERSISTENT TOTAL OCCLUSION AFTER MYOCARDIAL INFARCTION: ANALYSIS FROM THE OCCLUDED ARTERY TRIAL (OAT)**

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**Background.** In the Occluded Artery Trial, 2201 pts with an occluded infarct-related artery (IRA) were randomized to percutaneous coronary intervention (PCI) or medical treatment (MED). There was no difference in the primary endpoint of death, re-MI or heart failure (CHF). We examined the prognostic impact of pre-randomization stress testing.
**Methods.** Stress testing was required by protocol except for pts with single vessel disease and akinesis/dyskinesis of the infarct zone. Severe inducible