ID2: Monocytes contribute to neutrophil-dependent kidney injury in acute glomerulonephritis. 
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ID21: NOD1 sensing of Helicobacter pylori infection mediates processing of pro-interleukin-18 in gastric epithelial cells to maintain tissue homeostasis. 

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WM4: Human blastocyst-secreted microRNA-661 impairs endometrial receptivity via Mouse Double Minute 2 Homolog (MDM2). 
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Monocytes contribute to neutrophil-dependent kidney injury in acute glomerulonephritis.

Michaela Finsterbusch, Pam Hall, Anqi Li, A. Richard Kitching, and Michael J. Hickey.

Introduction: Glomerulonephritis is a leading cause of end-stage renal failure, a condition characterised by injurious inflammation of glomeruli. We recently observed that monocytes, similar to neutrophils, constitutively patrol glomerular capillaries, suggesting a role in immune surveillance of the glomerulus. However, the contribution of monocytes to the pathology of glomerulonephritis is unclear. Methods and Results: Using multiphoton and spinning disk confocal intravital microscopy, leukocyte behaviour was examined in the mouse kidney in a model of acute glomerulonephritis (using anti- glomerular basement membrane (GBM) antibodies). In this model, monocyte depletion reduced renal injury as assessed by albuminuria. Neutrophil recruitment, dwell time in glomerular capillaries and reactive oxygen species (ROS)-production by neutrophils were also diminished following monocyte depletion, suggesting a role for cross-talk between monocytes and neutrophils in mediating glomerular injury. Consistent with this, monocytes and neutrophils were seen to undergo interactions in the glomerular microvasculature, and these interactions were prolonged during anti-GBM Ab- induced inflammation. Notably, neutrophils that interacted with monocytes showed increased retention in the glomerulus and were more likely to produce ROS relative to non-interacting neutrophils. Moreover, renal monocytes, but not neutrophils, produced TNF during inflammation as detected via flow cytometry, and TNF was required for increased neutrophil dwell time and ROS production leading to renal injury. Conclusion: Collectively, our results suggest that monocytes mediate neutrophil recruitment and activation during glomerular inflammation via neutrophil- monocyte interactions and monocyte-derived TNF, triggering harmful neutrophil-dependent glomerular damage. Thereby, our data indicate a previously unrecognised intravascular inflammatory mechanism underpinning neutrophil-dependent glomerulonephritis.
**ID21**: NOD1 sensing of *Helicobacter pylori* infection mediates processing of pro-interleukin-18 in gastric epithelial cells to maintain tissue homeostasis.

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Persistent infection with *Helicobacter pylori* (HP) within the stomach causes chronic gastritis, an essential precursor to gastric cancer. Genetic polymorphisms in the IL18 gene strongly correlate with an increased risk of atrophic gastritis and gastric cancer, yet the activation and regulation of this cytokine in HP inflammation remain poorly understood. It has been reported that NOD- like receptor (NLR) family members, including NLRP1, 3, 6 and NLRC4 sense microbial components and mediate secretion of active IL-18 in innate immune cells through “inflammasome” assembly upon HP infection. By using bone-marrow chimeric mice, however, we identified non- hematopoietic cells as being the major source of IL-18 in the gastric mucosa of HP-infected mice and showed that loss of IL-18 in this cell compartment resulted in mucosal hyperplasia and increased acid mucins. Given that gastric epithelial cells (GECs) express the NLR protein NOD1, which detects Gram negative bacterial peptidoglycan and plays an important role in host immunity against HP infection, we further investigated the involvement of this receptor in HP-associated IL-18 responses. Here, we demonstrated that HP or HP-derived outer membrane vesicles induce mature IL-18 secretion in both mouse and human GECs. Gene knockdown showed that the HP-induced IL-18 secretion is driven by NOD1 receptor which interacts with caspase-1 via its caspase-activation recruitment domain (CARD), resulting in processing of pro-IL-18. Collectively, this is the first study, to our knowledge, reveals an unanticipated function of NOD1 signaling in posttranslational regulation of IL-18 in GEC and an important role for IL-18 in maintaining gastric mucosal homeostasis.
Human amnion epithelial cells modulate microglia to reduce cell death in a mouse model of perinatal brain injury.

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Introduction: Human amnion epithelial cells (hAECs) are clonogenic and have been proposed to reduce inflammatory-induced injury, via modulation of the immune response. Here, we investigate the effects of hAECs on microglia, the first immune responders to injury within the brain. We have generated a mouse model combining inflammation and hyperoxia, risk factors associated with perinatal brain injury. Methods: On embryonic day 16 we administered lipopolysaccharide (LPS), or saline (control), intra-amniotically to C57Bl/6J mouse pups. On post-natal day 0 (P0), LPS-treated pups were placed in hyperoxia (65% oxygen) and control pups in normoxia until P14. Pups were administered hAECs or saline intra-venously on P4. For co-culture experiments, primary microglia were isolated from P1-3 pup brains using magnetic sorting. To prepare conditioned media, hAECs (0.5 × 106) were seeded into T175 flasks with DMEM/F12 and 10% FBS, and cultured for 4 days. Results: At P14, relative to controls, LPS and hyperoxia pups had reduced body weight, increased global density of apoptotic cells, increased astrocytes in white matter, and increased activated microglia in the cortex and striatum but no change in total microglia density. hAECs increased body weight, reduced apoptosis and astroglia areal coverage in the white matter but paradoxically increased the density of total and activated microglia. We co-cultured primary microglia with hAECs, and found increased phagocytic activity, decreased microglia apoptosis and decreased pro-inflammatory activation markers. Conclusion: Our data demonstrate that hAECs can directly immunomodulate brain microglia, and offer promise that hAECs afford therapeutic utility in the management of perinatal brain injury.
PH11: Quality of life is poor for patients who require an interpreter: observations from the Australian Stroke Clinical Registry (AuSCR).

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Introduction: In multicultural Australia, there are patients with stroke are unable to speak or fully understand English. It remains unclear if patients with stroke who require an interpreter during hospitalisation receive poorer quality of care or experience worse outcomes. Method: Data from 45 hospitals participating in the Australian Stroke Clinical Registry from 2010-2014 were used. Health-related quality of life (HRQoL) was assessed using the EQ-5D-3L at 90-180 days. Propensity score matching of patients using age, sex and ability to walk on admission was undertaken to reduce selection bias. Between group comparisons were analysed using multilevel, multivariable regression. Results: Among 25,531 registrants, 1,049 (4.1%) required an interpreter. Compared to patients without interpreters, patients with interpreters were more often female (52% vs 46%), aged ≥75 years (68% vs 51%) and unable to walk on admission (71% vs 62%). Patients needing interpreters had greater access to stroke unit care (84% vs 77%; p<0.001) and were more often discharged on antihypertensive medication (72% vs 69%; p=0.03). After accounting for patient characteristics and stroke severity, patients requiring interpreters had comparable discharge outcomes to patients not needing interpreters, but reported poorer HRQoL (Coefficient -0.10; 95%CI -0.13,-0.07) including more problems with self-care (OR 2.08, 95% CI 1.55, 2.79), activity (OR:1.52; 95%CI 1.12, 2.07) and pain (OR:1.78, 95%CI 1.32, 2.38). Conclusion: Patients requiring interpreters reported poorer HRQoL 90-180 days after stroke despite receiving recommended care more often than those not needing an interpreter. Understanding the reasons for these differences may assist in providing better support for these patients.
CA3: The Inflammasome Adaptor ASC Promotes Tumour Cell Survival, Independent of Inflammation, in Gastric Cancer via IL-18

V. Deswaerte, P. Nguyen, T. Putoczki, B. Jenkins.

Introduction: Inflammasomes are key regulators of innate immunity, and despite their well-documented involvement in driving numerous chronic inflammatory disorders and autoimmune diseases, their role in inflammation-associated tumourigenesis remains ill-defined. Methods: We previously established the gp130F/F mouse model, which spontaneously develops gastric inflammation and intestinal-type gastric tumors. To investigate the role of the inflammasome in gastric cancer (GC), we assessed the expression and activation status of inflammasome associated components in tumours of gp130F/F mice. In addition, we genetically ablated ASC in gp130F/F mice. In order to correlate our findings to human disease, we used both human GC biopsies and cell lines. Results: Here, we reveal a pro-tumourigenic role for the key inflammasome adaptor ASC in GC. The genetic ablation of ASC reduced gastric tumour growth by augmenting cell death in the tumour epithelium, independent of hematopoietic- derived immune cells and chronic inflammation. Suppressed tumourigenesis was associated with reduced expression of IL-18, but not IL-1β, in gastric tumours, with targeted IL-18 deficiency in gp130F/F mice also decreasing tumour burden characterized by increased tumour cell death. Antibody-mediated blockade of IL-18 reduced human GC cell growth in vitro. Elevated IL-18 protein and ASC mRNA levels were observed in human GC tumour biopsies. Conclusion: Collectively, these findings reveal the ASC/IL-18 axis as a potential therapeutic target in GC.
CV11: Noninvasive CT-Derived Fractional Flow Reserve Based on Structural and Fluid Analysis.


Objectives: To describe the feasibility and accuracy of CT derived fractional flow reserve (FFR) to derive invasive FFR based on alternative boundary conditions.

Background: Techniques used to compute FFR based on images acquired from coronary-CT-angiography (CTCA) have been described. Boundary conditions are typically determined by allometric scaling laws. Alternatively boundary conditions can be derived from the structural deformation of coronary lumen though its accuracy remains unknown.

Methods: Forty-two patients (78 vessels) prospectively underwent 320-detector-CTCA and FFR. Deformation of coronary cross-sectional lumen and aorta, computed over diastole, was used to determine the boundary conditions based on Hierarchical-Bayes modelling. CT-FFR was derived using a reduced-order model performed on a standard desktop computer. First 12 patients (20 vessels) formed the derivation-cohort to determine optimal CT-FFR threshold to detect functional stenosis defined as FFR≤0.8, which was validated in the subsequent 30 patients (58 vessels).

Results: Derivation-cohort results demonstrated optimal CT-FFR threshold of 0.8, with 67% sensitivity, 91% specificity. In the validation-cohort, CT-FFR was successfully computed in 56/58 vessels (97%). Compared with CTCA, CT-FFR ≤0.8 demonstrated a higher specificity (87% vs 74%) and positive predictive value (74% vs 60%), with comparable sensitivity (78% vs 79%), negative predictive value (89% vs 88%) and accuracy (AUC 0.88 vs 0.77, P=0.22). Based on Bland-Altman analysis, mean intra-observer and inter-observer variability for CT-FFR was 0.02±0.05 and 0.03±0.06. Mean per-patient time for CT-FFR analysis was 27.07 minutes.

Conclusions: CT-FFR based on alternative boundary conditions and reduced-order fluid model is feasible, highly reproducible, convenient and may be accurate in detecting FFR≤0.8. Further validation is required in large prospective multicenter settings.
**CV32: Optimizing Care for Patients with Primary Aldosteronism: putting research into practice.**

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Background: Primary aldosteronism (PA) is the most common endocrine cause of hypertension, affecting up to 20% of those with resistant hypertension. Early diagnosis is crucial as it is potentially curable. Unfortunately very few centers have established protocols or expertise in its diagnosis and management, despite PA being recognized as a major public health issue. Objectives: We aim to develop center-specific guidelines for the diagnosis and management of PA and offer an evidence-based pathway of care for patients. Methods: An extensive literature review was performed on the diagnosis and management of PA. The information was collated into a center-specific protocol after consultation with all the stakeholders involved in the pathway of care, including endocrinologists, radiologists, chemical pathologists and endocrine surgeons. The PA protocol was introduced in January 2010. Subsequent patient outcomes were audited in 2016 to evaluate the impact of the protocol on clinical practice. Results: Introduction of the PA protocol led to a four-fold increase in the number of PA diagnostic procedures along with a 20% increase in the success rate of the procedures. The multidisciplinary approach to care has led to more standardized reporting by Diagnostic Imaging and Chemical Pathology. Analysis of patient outcomes has resulted in refinement of the protocol to optimize diagnosis. The significant increase in the number of PA diagnoses has necessitated the establishment of a dedicated Endocrine Hypertension Clinic at Monash Health. Conclusion: An evidence-based center-specific protocol developed from PA research has been successfully implemented in clinical practice, leading to increased diagnoses and improved patient care.
**WM4: Human blastocyst-secreted microRNA-661 impairs endometrial receptivity via Mouse Double Minute 2 Homolog (MDM2).**

Amy L Winship 1, Amanda Ton 1, Michelle Van Sinderen 1, Ellen Menkhorst 1, Carly Cuman 1 and Evdokia Dimitriadis 1,2:

**Introduction:** Synchronous human embryo development and endometrial receptivity are essential for pregnancy success. However, understanding of the critical factors that regulate blastocyst-endometrial interactions is limited. Human IVF blastocysts that fail to implant secrete elevated levels of microRNA (miR)-661, which is taken up by the endometrium to alter gene expression and adhesion. We investigated mechanisms of miR-661 regulation of receptivity in women. MiR-661 regulates mouse double minute homolog 2 (MDM2) in breast cancer cells, although MDM2 has not been studied in the endometrium. Methods: We immunolocalized MDM2 in fertile and infertile endometrial tissue during the receptive phase of the menstrual cycle (n=8/group). Primary human endometrial epithelial cells (HEECs) and Ishikawa (endometrial epithelial cell line) were transfected with miR-661 mimic (synthetic miR) or control. The effect on MDM2 expression was determined (n=4/group). To model endometrial-blastocyst adhesion, we investigated the effect of HTR8/SVneo (trophoblast cell line) spheroid adhesion to Ishikawa cells, or HEECs following MDM2 knockdown by siRNA (n=3/group). Results: MDM2 localized to the endometrial glandular and luminal epithelium (site of blastocyst attachment) during the receptive phase. MDM2 immunostaining was decreased in endometrial epithelium from infertile versus fertile women (p<0.05). MiR-661 down-regulated MDM2 in Ishikawa and HEECs. MDM2 knockdown in Ishikawa and HEECs reduced HTR8/SVneo spheroid adhesion to both (p<0.05). Conclusion: These findings advocate that human blastocyst-secreted miR-661 alters endometrial receptivity via MDM2. This highlights a potential new mechanism by which human blastocysts affect endometrial receptivity. This has important implications in developing biomarkers for embryo implantation potential and treatments of implantation failure.