**Bhargava, V**[1], Rana, DS[1], Jain, M[2], Kumar, D[3], Bhalla, AK[1], Gupta, A[1], Gupta, A[1], Malik, M[1]

[1]Sir Gangaram Hospital, Nephrology, New delhi, India; [2]Sir Gangaram Hospital, Genetic medicine, New delhi, India; [3]Sir Gangaram Hospital, Research Department, New delhi, India

**Introduction:** The clinical impact of anti-HLA antibodies is one of the major area of research in renal allograft transplantation. Luminex Platform has emerged as the favoured technology for detection of HLA antibodies in addition to the CDC crossmatch (XM). Luminex DSA crossmatch has been recently introduced in India for detecting antibodies that are missed by CDC XM. We present our experience with the Luminex based DSA crossmatch test done in the recipients who underwent transplantation at Sir Ganga Ram Hospital in the year 2013-14 with one year post transplant follow up. The objective of the study was to evaluate the impact of the pre-Tx DSAs detected by Luminex crossmatch on the clinical outcome of the renal graft over a period of one year

**Methods:** We began performing DSA (donor specific antibody) monitoring protocol by Luminex in the year 2013 at our center. The present study includes patients from January 2013 to December 2013 with one year follow up post kidney transplant. Pre- transplant sera from 46 renal transplant recipients with a negative CDC crossmatch were assessed for donor-specific antibodies (DSA) detection on Luminex Platform using Lifecodes DSA kit (Immucor). The serum samples with DSA (HLA-Class I or II or both) of MFI (mean fluorescent intensity) value more than 500 was considered to be positive. The results were then correlated with the clinical outcomes of the renal allograft.

**Results:** SAs were found in 11 out of 46 recipients (23.9%). Of the eleven DSA positive patients, 3 patients (27.27%) developed acute graft rejection. All these 3 patients had positive C4d staining in their biopsies and the MFI value of the DSA on Luminex platform was found to be more than 1000. The remaining 8 DSA positive patients showed no rejection and had stable graft function. The MFI value of the DSAs in these patients ranged from 500-1000. All the 35 DSA negative patients (76.1%) were also having stable graft function in one year follow up. Hence, AMR was more frequent in the DSA positive group than in DSA negative group. outcome of the renal graft over a period of one year.

**Conclusions:** The present study evaluates the importance of Luminex DSA crossmatch test in detecting the donor specific HLA antibodies over the CDC crossmatch. There was a higher incidence of AMR in patients with pre-transplant DSA despite a negative CDC crossmatch. The present study clearly establishes that the Luminex DSA crossmatch is helpful for predicting post transplant graft outcome or rejection. The laboratory cut off value of the MFI for positive DSA was increased from 500 to 1000. We suggest that DSA MFI value above 1000 should be considered for further evaluation by Single antigen bead assay (SAB) by Luminex. However, the clinical impact of the pre-Tx DSAs detected by Luminex techniques has to be fully evaluated in terms of graft survival and more retrospective studies with larger sample size.

## P14

## MIF IS AN ENDOGENOUS FIBROSIS LIMITING FACTOR IN PROGRESSIVE KIDNEY DISEASES

Djudjaj, S[1], Martin, IV[2], Nothofer, N[1], Buhl, ME[1], Floege, J[2], **Boor, P**[1]

[1]RWTH Aachen University, Institute of Pathology, Aachen, Germany; [2]RWTH Aachen University, Department of Nephrology, Aachen, Germany

**Introduction:** Renal fibrosis is the underlying process and the common end-point of progressive kidney diseases. A multitude of factors and processes were found to promote and aggravate renal fibrosis, but our understanding of endogenous factors limiting fibrosis is limited.

**Methods:** Here we analyzed the role of macrophage migration inhibitory factor (MIF), a pleiotropic proinflammatory cytokine, in animal models of renal tubulointerstitial fibrosis and inflammation.

**Results:** We show that MIF expression is reduced in murine and human renal tubulointerstitial fibrosis. MIF inhibition using gene knock-out, neutralizing antibodies or a small-molecule inhibitor aggravated fibrosis and worsened parameters of renal function, while treatment with murine recombinant MIF abrogated renal fibrosis. This effect was consistent in three distinct models of renal fibrosis, i.e. those induced by obstruction, ischemia-reperfusion or toxin, and was also effective when treatment was initiated in already established fibrosis. Bone-marrow chimeras showed that local but not bone-marrow derived MIF was involved. Mechanistically, MIF reduced the expression of chemokine MCP-1 in tubular cells and reduced renal inflammatory infiltrates already early in disease.

**Conclusions:** Taken together, we identified a hitherto unappreciated role of MIF as an endogenous factor limiting renal tubulointerstitial fibrosis via limiting renal inflammation. Our data raise important safety concerns regarding the envisaged use of MIF inhibition as a treatment for inflammatory diseases.

## P15

## THE EFFECT OF PEPTIGYLARGININE DEIMINASE 4 INHIBITOR ON MPO-ANCA PRODUCTION IN MOUSE MODEL

**Kusunoki, Y**[1], Nakazawa, D[1], Hattanda, H[1], Miyoshi, A[1], Shida, H[1], Masuda, S[2], Tomaru, U[3], Nishio, S[1], Atsumi, T[1], Ishizu, A[2]

[1]Hokkaido University Hospital, Department of Internal Medicine II, Sapporo, Japan; [2]Hokkaido University, Faculty of Health Sciences, Sapporo, Japan; [3]Hokkaido University Graduate School of Medicine, Department of Pathology, Sapporo, Japan

**Introduction:** MPO-ANCA-associated vasculitis (MPO-AAV) is a systemic small vessel vasuculitis that involves preferentially the kidneys. It has been shown the pathogenic role of MPO-ANCA in MPO-AAV. Neutrophil extracellular traps (NETs), which are released from neutrophils activated by microorganisms, are composed of net-like chromatin fibers and antimicrobial proteins, such as MPO. The activated neutrophils die in due course with the formation of NETs. During the NET formation, histones bound chromatin fibers are citrullinated by peptigylarginine deiminase 4 (PAD4), and consequently the chromatin fibers are decondensated. Thus, PAD4 plays a pivotal role in the NET formation. Although NETs are essential for elimination of microorganisms, excessive formation of NETs has been shown to be implicated in the MPO-ANCA production. The aim of this study is to determine that inhibition of PAD4 can suppress the NET formation and MPO-ANCA production in vivo.

**Methods:** According to our previous report, NETs were induced in peripheral blood neutrophils derived from healthy donors ($1\times106$/ml) by stimulation with 20 nM phorbol myristate acetate (PMA) with or without 20 μM anti-thyroid drug, propylthiouracil (PTU) for 2 hours at 37 °C. The effect of PAD4 inhibitor, 200 μM Cl-amidine, on the in vitro NET formation induced by 20 nM PMA