

P078

Lymphoid Blast Crisis of Chronic Myeloid Leukaemia in a Jehovah Witness Patient

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Lymphoid blast crisis of Chronic Myeloid Leukaemia (CML) occurs in about 30% cases and is generally reported to have a better prognosis than myeloid blast crisis. The presence of the b2a2 fusion transcripts and 5' M-bcr breakpoints also confers an improved outcome. The case is of a 57 year-old woman with Hereditary Angioedema presenting with a blood picture in keeping with myeloproliferative disease. Bone marrow examination was diagnostic for chronic phase Chronic Myeloid Leukaemia. In the case presented, a treatment regimen, no longer routinely used for CML (busulfan therapy), rather than the current imatinib mesylate therapy, undoubtedly resulted in rapid progression to blast crisis. The use of busulfan in chronic phase CML patients should be limited to those patients who are intolerant of, or resistant to other therapy.

The lymphoid blast crisis appeared 11 months after diagnosis of the CML. The patient was referred to a Haematologist and treated with vincristine, Glivec and prednisone. She was not given standard therapy which would have required substantial blood product support. The therapy caused a prolonged period of severe pancytopenia.

Erythropoietin (EPO) was used for the anaemia. The patient had a brief haematological remission but 3 months later she relapsed and succumbed.

The ethical issues of treatment which normally necessitates the extensive use of blood product support are highlighted in this case of a Jehovah Witness patient. Refusal to receive blood components greatly affects treatment options. The course of this patient's disease would undoubtedly have been quite different had she received current therapy (Imatinib mesylate) for CML at diagnosis.

No conflict of interest to disclose

P079**Monosomy 7 in Paediatric Patients: A Case Series With 5 Different Haematological Presentations****Helen Weston**, Catherine McCarthy, Bronwyn Williams*Pathology Queensland, Royal Brisbane & Women's Hospital, Herston, Queensland, Australia***Aim**

Monosomy 7 and deletions of 7q occur in a variety of myeloid disorders, particularly AML and MDS, disorders which are more commonly found in adults than children. We aim to report 5 paediatric patients with haematological disorders, associated with a complete or partial monosomy 7.

Methods

Consecutive patients with patients with complete or partial monosomy 7 presenting to our institution over a 12 month period were identified. Data was collected regarding clinical features, bone marrow morphology and conventional cytogenetics plus FISH analysis.

Results

Five patients were identified, with ages 1 to 6 ½ years, 3 males and 2 females. Diagnoses were AML in 2 patients (one de novo AML with multilineage dysplasia and one with trisomy 21, and one patient each with MDS, JMML and aplastic anaemia. The patient with de novo AML had persistent disease following induction phase II, and underwent an unrelated umbilical cord in CR1. This patient relapsed at day 250 and died shortly afterwards. The patient with trisomy 21 AML had an additional ring isochromosome 7 and trisomy 8, and is in CR and completing chemotherapy. The patients with JMML and MDS have been successfully allografted and remain alive in CR1 at day 70 and 160, respectively. The patient with JMML had a small ring chromosome derived from chromosome 7, while the patient with MDS had Wiskott Aldrich Syndrome, confirmed on WASP gene sequencing. The patient with aplastic anaemia was treated with ATG and cyclosporin A, and remains in a complete haematological and cytogenetic remission after 9 months of followup.

Conclusion

Our 5 cases demonstrate that in addition to the more common presentation with AML or MDS, monosomy 7 /7q- can be associated with more unusual presentations in childhood, including aplastic anaemia and Wiskott-Aldrich syndrome.

No conflict of interest to disclose

A296

P080

Recurrent Transfusion-Related Spontaneous Remission of Acute Myeloid Leukaemia

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Aim

Spontaneous remission of acute myeloid leukaemia (AML) has been reported on a number of occasions – usually in the context of infection or blood transfusion. We report experience with a recent case and review previous published cases in an attempt to further delineate this relationship and postulate possible underlying mechanisms.

Method

We analysed circumstances surrounding induction of remission in our index case and performed a literature search using PubMed with keywords ‘spontaneous remission’, ‘acute myeloid leukaemia’ and ‘transfusion’.

Results

An asymptomatic 81y.o. female presented with isolated neutropenia. Bone marrow biopsy (BM) revealed myelodysplastic features consistent with Refractory Anaemia with Excess Blasts (RAEB-I). Seven months later, she developed progressive pancytopenia. Repeat BM revealed progression to AML with normal cytogenetics. Transfusion of two units of non-leucodepleted and non-irradiated red blood cells (RBCs) was complicated by a febrile reaction. Within weeks and without other intervention, peripheral blood counts normalised. Two months later, the pancytopenia recurred. After further RBC transfusion and associated febrile reactions, peripheral counts again normalised within weeks and repeat BM demonstrated leukaemic remission with no excess blasts. She has received no other treatment to date. Five months after the most recent transfusions, she has evidence of myelodysplasia with mild thrombocytopenia and leucopenia but no significant peripheral blast count. In the search undertaken, approximately 33 cases of spontaneous remission from AML have been published since 1980 where patients have invariably received a transfusion or presented with infection. Mean duration of remission was approximately seven months. Only three remissions occurred in the setting of myelodysplasia.

Conclusion

We conclude a possible immune basis for these remissions – due to the transfusion of immunocompetent anti-leukaemic leucocytes with the red cells, cytokine induction by transfusion or infection, or the development of other anti-leukaemic cellular or humoral immune responses induced by infection or transfusion.

No conflict of interest to disclose

A297

P081

Two Fatal Cases of Central Nervous System Scedosporiosis Occurring After Neutrophil Recovery Following Induction Chemotherapy for Acute Leukemia**Michelle Spanevello**, Glen A Kennedy, Kirk Morris*Department of Haematology, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia*

Scedosporium prolificans is an emerging opportunistic pathogen which disseminates quickly and is often refractory to therapy in the immunocompromised patient. It is being increasingly reported in patients with acute leukemia who are neutropenic, and involvement of the central nervous system (CNS) in these situations is almost universally fatal. We describe two recent cases of CNS Scedosporidiosis manifesting after neutrophil recovery which were ultimately fatal despite early identification and institution of appropriate therapy.

Case 1

A 28 year old female with acute myeloid leukemia developed rapidly progressive unilateral chemosis and visual loss due to endophthalmitis on Day 32 of her induction chemotherapy. The onset of symptoms occurred five days following achievement of a normal neutrophil count and whilst remaining on empiric liposomal amphotericin. Despite immediately changing to voriconazole at the onset of visual symptoms and subsequently introducing terbinafine when *Scedosporium prolificans* was identified from blood cultures, she suffered multiple and inevitably fatal intracerebral haemorrhages associated with ruptured mycotic aneurysms caused by a diffuse cerebral vasculitis.

Case 2

A 52 year old female received one cycle of HyperCVAD for precursor B-cell acute lymphoblastic leukemia. Approximately seven days after recovery of a neutrophil count to $> 1.0 \times 10^9/L$, she complained of a severe generalised headache. CSF and blood cultures demonstrated *Scedosporium prolificans*. Despite immediate commencement of voriconazole and terbinafine, she developed initial focal limb weakness followed by progressive loss of consciousness and death within a week of CNS symptoms.

Conclusions

CNS Scedosporiosis in immunocompromised patients is almost universally fatal. Combination voriconazole and terbinafine is the currently recommended antifungal strategy. To our knowledge diffuse cerebral vasculitis with mycotic aneurysm formation and haemorrhage has not been described. The onset of clinical manifestations occurring after neutrophil recovery in both cases was unexpected. Increased awareness of this pathogen is encouraged.

No conflict of interest to disclose

A298

P082

CD4+/CD56+ Haematodermic Tumour in Transformation?

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An 89 year old lady, previously well presented with a two week history of a rapidly growing cutaneous violaceous plaque on her mid-back region. This measured at 10x15cm when reviewed. She also described multiple small circumferential subcutaneous pink nodules up to 1cm in diameter appearing throughout her body over the same period. Skin biopsy revealed a dermal infiltrate with sheets of immature cells with immunohistochemistry profile of CD3-, CD4(weak), CD56 (weak), CD43+ and CD117-. FBC and E/LFTs were normal. BMAT was not involved morphologically or by flow cytometry. Haematodermic neoplasm was diagnosed.

Given her age she was treated with localised radiotherapy and two cycles of chlorambucil and prednisolone with good response. Ten weeks after her initial diagnosis a routine FBC showed leukaemic dissemination. Her smear revealed 80% "large blastic cells that appear to be of monocytoid origin". The immunophenotype was CD4+, CD56+, CD13+, CD14+, CD11b+, CD33+, CD34-, cMPO-, CD117- and CD3-. She died 4 days later.

CD4+/CD56+ haematodermic neoplasms (HDN) are uncommon; designated cutaneous lymphomas under the revised 2005 WHO-EORTC classification. Previously known as blastic NK cell lymphoma, they are more recently termed early plasmacytoid dendritic cell leukaemia/lymphoma. They are highly aggressive with a poor prognosis (median survival 12-14 months), usually presenting with skin lesions, rapid nodal and marrow involvement with leukaemic dissemination and progression to AML. This close relationship to acute myelomonocytic and monoblastic/monocytic leukaemia (FAB -M4/M5), is evident with up to 30% of this subgroup expressing CD56.

The patient had morphological features of transformation to AML with myelomonocytic differentiation (CD11b, CD13, CD14, CD33) but lacked the key myeloid antigens for a definitive diagnosis of AML (cMPO and CD117).

This is an unusual case of apparent/incomplete transformation of HDN to AML (described by Craig et al. In *Blood* April 15, 2008) It is uncertain whether repeat immunophenotyping, several days later (had the patient survived) would yield a similar finding or complete transformation to AML.

No conflict of interests disclosed

A299

P083

Philadelphia Chromosome t(9;22) in AML with Additional Cytogenetic Abnormalities: A Report of 2 CasesDavid Yeung¹, Mary Suter², Peter Tan¹, John Moore¹¹ Department of Haematology, St Vincent's Hospital, Darlinghurst, NSW, Australia.² Sydpath, Darlinghurst, NSW, Australia**Aim**

We describe the characteristics of 2 patients with AML, having the Philadelphia chromosome t(9;22) either as a secondary abnormality or part of a complex karyotype.

Case 1

A 66 year old man presented with fever, sweats, bone pain and confusion from hypercalcaemia. Initial pathology results showed haemoglobin of 114gm/L; Platelet of $57 \times 10^9/L$; WCC of $6.8 \times 10^6/L$ with 4% blasts. Marrow aspirate was consistent with a diagnosis of AML. Karyotype showed an apparent terminal deletion of 9q, a derivative of (22) t(9;22), and a t(12;13). A single BCR-ABL fusion gene was found on FISH. Induction therapy with 7-3-7 and Imatinab was given; followed by 2 consolidations with 5-2-5. Molecular remission (BCR-ABL) was achieved, but last only 6 months. The same clone was detected at relapse.

Case 2.

A 24 year old man presented with cervical lymphadenopathy. Pathology results showed haemoglobin of 90gm/L, WCC of $62 \times 10^6/L$ with 75% blasts, monocytosis of $7.4 \times 10^6/L$, and platelet count of $26 \times 10^9/L$. Marrow aspirate showed AML, (Monoblastic variant with Eosinophilia). Cytogenetics revealed inv16 in all cells and an additional t(9;22;19) in 50% of cells, confirmed by PCR and FISH as p190 BCR-ABL. The patient was commenced on a Fludarabine/Cytarabine combination chemotherapy (ALLG AML13 protocol), later switching to 7-3 after developing pneumonitis to fludarabine; 2 consolidations with 5-2 were given. He relapsed 3 months after molecular remission with respect to both BCR-ABL and CBFb. At relapse, inv(16) was detected without evidence of t(9;22;19) on cytogenetics or FISH. The patient remains disease free after a double-cord cell stem cell transplant.

Discussion

These two cases describe the presence of BCR-ABL mutation in AML. Anecdotally, this mutation appears to confer a bad prognosis. In Case 1 the mutation appears to be part of a complex karyotype, with the BCR-ABL possibly playing a major role in pathogenesis. In the Case 2 the BCR-ABL mutation in the malignant clone was probably secondarily acquired in clonal evolution, being only present in 50% of leukemic cells at outset. The patient was initially classified as CBFb and treated as good risk. However, the presence of BCR-ABL may have played an enabling role to proliferation even though it was absent at relapse. This appears to be the only case reported of p210 BCR-ABL with inv(16).

No conflict of interest to disclose

A300

P084

Secondary Donor-derived Burkitt Leukemia following Allografting for Aplastic Anaemia

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Case Report

The patient presented at the age of 17 with a one year history of lethargy and was subsequently found to have aplastic anaemia. Primary treatment was with sibling allogeneic transplant, completed in October 2000. Conditioning was with cyclophosphamide and ATG and GVH prophylaxis was with cyclosporine and methotrexate. She remained on cyclosporin A until May 2003 for GvHD. Whilst remaining asymptomatic, routine blood tests for an outpatient follow-up in November 2003 showed a leukoerythroblastic blood film. The ensuing bone marrow biopsy showed a population of cells expressing CD10, CD19 and CD20 with Kappa light chain restriction and the characteristic morphology of Burkitt leukaemia. Cytogenetics identified the t(2;8)(p12;q24) abnormality which is rare, but consistent with Burkitt type acute lymphoblastic leukaemia. This clone was donor-derived with the XY karyotype. The patient completed six cycles of Hyper CVAD and has remained in remission for over four years.

Post-transplant lymphoproliferative diseases occur most commonly in the setting of ongoing immunosuppression and the majority are B cell non-Hodgkin lymphomas attributable EBV infection. Burkitt leukaemia has rarely been reported in the post-transplant setting and only twice in the donor clone following allogeneic stem cell transplantation. Such cases have been associated with concurrent EBV infection.

At the time of diagnosis with Burkitt leukaemia the patient was found to have no serological evidence of EBV (with both IgG and IgM undetectable) and EBV could not be detected by PCR at that time. The donor had serological evidence of previous EBV infection with IgG but not IgM at the time of donation and in November 2003 did not have detectable EBV by PCR.

In summary, this case represents a rare post-transplant lymphoproliferative disease that occurred in the donor clone following an allogeneic stem cells transplant in the absence of significant immunosuppression and without evidence of overt EBV infection.

No conflict of interest to disclose

A301

P085**Air Filtration in Stem Cell Transplant Units: Is it Always Necessary?****Catherine Wood¹**, Leonie Johnson¹, Barry Anthony¹, Mark Jones², Michael Humble² & John Carter¹¹ *Wellington Blood and Cancer Centre, Wellington, New Zealand* ² *Microbiology Department, Wellington Hospital, Wellington, New Zealand*

Infections are a significant cause of morbidity and mortality in recipients of allogeneic haematopoietic stem cell transplants. International guidelines exist regarding prevention and treatment of bacterial, viral and fungal infections in this setting. The use of HEPA filtered air is often mandated by best practise guidelines to reduce infection, especially *Aspergillus* and other spore born fungi. The evidence basis for this is debatable and implementation of these recommendations is often expensive and they take little account of geographical variance in the incidence of such organisms.

The Wellington Transplant Unit has historically had a very low incidence of fungal infections. Recently the unit has been exposed to major dust contamination from building demolition and rebuilding on the hospital campus. To obviate this threat the transplant unit has nursed patients in newly commissioned HEPA filtered rooms. This project has been prospectively monitored with environmental surveillance cultures and recording of patient infections. Data will be presented showing a very low environmental contamination and a low patient infection rate. This challenges the universal recommendation for the need for protected rooms. This conclusion is further supported by a care model that emphasises early patient discharge to a day care unit which does not have protected rooms.

No conflict of interest to disclose

A302

P086

Extramedullary Relapse of Acute Myeloid Leukemia Post-Allograft

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Aims

The incidence; characteristics and prognosis of extramedullary (EM) relapse of acute myeloid leukemia (AML) post-allogenic transplant is investigated, and we evaluate the potential of PET as a tool in diagnosis.

Methods

Cases were retrospectively reviewed from a single transplant centre of patients having EM relapse of AML post allogenic transplantation from 1997-2007.

Results

Out of 72 cases of AML who had allogenic transplant, 6 cases (8%) had EM relapse. Initial diagnosis age ranged between 19 and 48 years. Five patients were in disease relapse at the time of transplant and one in complete remission. After myeloablative conditioning all received peripheral blood stem cell transplant. Five patients had GVHD prophylaxis with methotrexate and cyclosporine and one had tacrolimus. Half the patients (3) had acute GVHD grade 2-4. Five had extensive chronic GVHD and one had no chronic GVHD. Time to relapse at extramedullary sites ranged from 15 months to six years. All had normal bone marrow at the time of relapse. Sites of extramedullary relapse included testis, axillary soft tissue, epidural space, CNS, lymph nodes, retro-orbital space, bone, chest wall and stomach. PET showed high uptake in areas of EM relapse. All PET positive cases were corroborated histologically. Only one patient had subsequent bone marrow relapse. Currently, two patients are alive (11 and 20 months post EM relapse), four died after 5, 17, 20 and 28 months post EM relapse.

Conclusions

EM relapse post allograft is occurring in 8% of AML allografts and with a very poor long term prognosis. EM relapse has widespread sites of presentation with a wide range in time to relapse. It appears more common in patients who had transplant in relapse and occurs despite the development of GVHD. This study also shows that PET can aid in monitoring relapse and treatment response.

No conflict of interest to disclose

A303

P087

Post-Transplant Thrombotic Microangiopathy: Impact of New Diagnostic Criteria on Diagnosis

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Aims

To determine the sensitivity of the newly proposed Bone Marrow Transplant Clinical Trials Network (BMT-CTN) and International Working Group (IWG) diagnostic criteria for transplantation-associated thrombocytopenic microangiopathy (TA-TMA).

Methods

Patients with TA-TMA occurring post-allogeneic transplantation between December 2001 and June 2008 were identified from an institutional data-base. Haemoglobin and platelet levels, changes in blood and platelet transfusion requirements, LDH level, % fragmented cells, Coombs test results, serum haptoglobin, serum creatinine and neurological complications at time of TA-TMA diagnosis were determined retrospectively from review of patient records. Patients were then classified as per BMT-CTN and IWG TA-TMA criteria as previously published (BBMT 2005; 11: 571; Haematologica 2007; 92: 95).

Results

In total, 15 patients with TA-TMA were identified, representing 3% of all allogeneic transplants performed at our institution during the review period. A significant proportion of patients failed to meet both newly proposed criteria. The main limitation of the BMT-CTN criteria was the need for concurrent renal and / or neurological dysfunction to be manifest at TA-TMA diagnosis, which was present in only 73% of our patient cohort. For the IWG criteria, the main limitation to TA-TMA diagnosis was the requirement for a level of schistocytosis >4%, which was definitively present in only 27% of our patients. Median overall survival for the whole cohort was 79 days (range 5-1845 days), and was similar between patients who met or failed to meet either the BMT-CTN or IWG criteria ($p=0.5$ and $p=0.78$ respectively).

Conclusions

Our experience suggests that both the BMT-CTN and IWG diagnostic criteria are relatively insensitive, with both potentially misdiagnosing a significant percentage of TA-TMA. Given this diagnostic insensitivity, we caution against widespread adaptation of either the BMT-CTN or IWG criteria as they currently stand.

No conflict of interest to disclose

A304

P088**Is a Second Transplant Worthwhile in Patients with Haematological Malignancies Relapsing After a Previous Autologous Transplant?**

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Introduction

Autologous stem cell transplantation (ASCT) is intended as a single treatment procedure. Where significant remission is achieved, the question arises as to the benefit of a second ASCT at subsequent relapse.

Method

Between 1995 and 2007 inclusive, our institution carried out a total of 243 stem cell transplants (counting "tandem transplants" as a single procedure). Of the 243, 7 were true second transplants. The interval between first and second transplants ranged from 1.9-7.6 years. Patients' ages at first transplant ranged from 41-51 and at second transplant 42-55 years. Three had multiple myeloma and 4 had non-Hodgkin lymphoma (NHL). Six were males. Two patients were given transplants with blood stem cells that had been collected and cryopreserved prior to the first transplant; in the other cases the cells were collected prior to the second procedure.

Results

Comparison of stem cells reinfused for the first and second transplants showed median CD34+ cells of 2.9 and 2.0 x 10⁶/kg respectively. Median neutrophil recovery (0.5 x 10⁹/l) was 9.9 days and 10.4 respectively. However platelet engraftment ($\geq 20 \times 10^9$ litre) had a tendency to be delayed after second transplant (10.1 and 19.3 days respectively) with one patient remaining platelet transfusion dependent at 255+ days following his second reinfusion. Overall survival from first transplant ranged from 1250 to 4178 days and from the second transplant 214 to 2031 days, with six of the seven patients still alive on 30 June 2008.

Conclusion

In cases where long-term remission has been achieved by initial ASCT, a second ASCT can be considered of value for management of patients with relapsed haematological malignancies.

There is no conflict of interest to disclose

A305

P089

Should Immunoglobulin Therapy (IVIg) be used as Prophylaxis against Infection in Multiple Myeloma (MM) Patients undergoing Haemopoietic Stem Cell Transplantation (HSCT)?

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Aim

We assessed the effectiveness of IVIg use in the peritransplant period in preventing sepsis in MM patients who had undergone HSCT.

Method

A retrospective review of consecutive patients with MM who underwent HSCT between January 2000 and December 2007. We looked at the timing of IVIg administration, febrile episodes and microbiological isolates from Day -7 to Day 30 post HSCT.

Results

208 HSCT were performed on 195 patients (137 male), median age 59 (range 34 to 79 years). 94% had associated hypogammaglobulinaemia at HSCT. 177 patients (91%) had ≥ 1 febrile episode requiring blood cultures. IVIg administration was based on clinical decisions.

The patients were allocated into 4 groups based on IVIg administration:

PRE Patients received IVIg at any time pretransplant.

PERI Patients received peritransplant IVIg (Day -7 to Day 30 post HSCT)

PREPERI Patients received IVIg pre and peritransplant.

NIL Patients did not receive IVIg.

Group	Patients	Positive Blood Cultures	Gram + organisms	Gram -ve	Fungal +
PRE	19	9 (47%)	10	4	0
PERI	69	40 (58%)	28	11	1
PREPERI	52	21 (40%)	12	6	2
NIL	55	23 (42%)	15	11	0

Culture positivity for bacterial or fungal infections was not increased in those patients not receiving IVIg replacement at any time before or during their HSCT.

Conclusion

Despite the influence of possible selection bias in this population this review suggests that IVIg in the pretransplant, peritransplant, or in both periods does not reduce the number of infective episodes for HSCT in MM.

No conflict of interest to disclose

A306

P090

Pegfilgrastim Without Chemotherapy Effectively Mobilises PBPC Cells – Early Results of a Single Centre Study

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Aim

Pegfilgrastim (PEG-f) has proven an effective Peripheral Blood Progenitor Cell (PBPC) mobilising agent when administered the day after chemotherapy for patients with myeloma and lymphoma. We have explored the use of PEG-f without chemotherapy to mobilise PBPCs. We compared the mobilisation kinetics of PEG-f to an historical group receiving traditional filgrastim without chemotherapy.

Method

To date, 13 patients who would otherwise be eligible for filgrastim-alone mobilisation were mobilised with PEG-f and compared to 22 patients mobilised with filgrastim. All patients were not actively receiving chemotherapy and were collected for the purpose of long-term PBPC storage ('rainy day' collection). The 13 patients received a 12ug dose of PEG-f. The 22 patients mobilized with filgrastim received daily subcutaneous injections of 10µg/kg filgrastim. For all patients, monitoring commenced on Day 4, three days following initial dose of filgrastim or PEG-f.

Result

We found the median peripheral blood (PB) CD34 count on the first day of monitoring (day 4) was greater for PEG-f mobilisation ($p=0.052$). On days 5, 6 & 7, differences were not statistically significant, although median on each day was greater for Peg-f than filgrastim.

Day of collection	Median PB CD34 ⁺ (cells/ul)	
	PEG-f	Filgrastim
4	36.15	15.75
5	33.9	28.85
6	20.75	17.95

The apheresis collection data demonstrated a pattern similar to the PB CD34⁺ counts. The median CD34⁺ cells collected on any given day was 1.48×10^6 /Kg for

patients mobilised with PEG-f and 1.24×10^6 /Kg for patients mobilised with filgrastim. All 13 patients mobilised with PEG-f harvested a total $\geq 2 \times 10^6$ CD34⁺/Kg, 10 of whom (77%) harvested a total $\geq 4 \times 10^6$ CD34⁺/Kg. Of the patients mobilised with filgrastim, 16 of 22 (72%) harvested a total $\geq 2 \times 10^6$ CD34⁺/Kg and 13 of 22 (59%) harvested a total $\geq 4 \times 10^6$ CD34⁺/Kg.

Conclusion

We conclude that PEG-f without chemotherapy effectively mobilises PBPCs. The study is ongoing.

No conflict of interest to disclose

A307

P092**Bilateral Humeral Head Osteonecrosis Following Cladribine Therapy for Hairy Cell Leukaemia (HCL)****A Bazargan, S Opat***Department of Haematology, Monash Medical Centre, Clayton Road, Clayton, VIC 3168***Background/Aim**

Osteonecrosis of the femoral or humeral heads is a recognised complication of chemotherapy for lymphoma with corticosteroid containing regimens and following radiotherapy. Its occurrence in hairy cell leukaemia and in the absence of corticosteroids is rare. We report a case of bilateral humeral head avascular necrosis following cladribine chemotherapy for hairy cell leukaemia and review the literature for further cases.

Result

A previously healthy 45 year old man presented with pancytopenia and was subsequently diagnosed with HCL on bone marrow biopsy. He was treated with a seven day course of cladribine, however at six months a repeat bone marrow biopsy showed a small focus of persistent disease. He received a second, seven day course of cladribine, which was uneventful until the final day when he developed right sided shoulder pain. A shoulder X-ray did not reveal any abnormalities, however a bone scan showed striking increase in radiotracer uptake in both humeral heads consistent with osteonecrosis. He was managed conservatively and made a full recovery. He did not receive steroids or radiation and there was no history of trauma, excess alcohol ingestion, hyperlipidemia or smoking. A PubMed search identified no previous reports of osteonecrosis associated with cladribine and a single case of osteonecrosis occurring several years after therapeutic splenectomy for HCL.

Conclusion

The pathogenesis of osteonecrosis in HCL and other lymphoproliferative disorders is uncertain with current opinion suggesting a contribution from drugs, metabolic factors, altered blood supply, increased intraosseous pressure, mechanical stresses and the presence of tumour. Ninety percent of cases are caused by corticosteroid therapy and excess alcohol ingestion. It has been suggested that osteonecrosis may occur at sites of tumour involvement; whether the humeral heads were involved with HCL in this patient is unknown. The close temporal relationship of cladribine therapy and the development of symptoms suggest a causal relationship.

*No conflict of interest to disclose***A308**

P093

Haemophagocytic Lymphohistiocytosis (HLH) Following Elective Percutaneous Coronary Intervention (PCI), A Case Report and Literature Review

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Background

HLH is a rare, often fatal disorder characterised by dysregulation of natural killer and T-cell function, resulting in cytokine overproduction and activation of macrophages with haemophagocytosis. We report a case of HLH occurring shortly after elective PCI, possibly implicating it as a trigger for this episode.

Aim

To examine the association of HLH with PCI and clopidogrel.

Methods

Case report and systematic literature review.

Results

A 74 year old woman with a history of ANCA positive vasculitis, well controlled on cyclophosphamide and prednisolone, presented for elective PCI for stable angina. FBE was normal on admission. A loading dose of clopidogrel was administered pre-procedure then 75mg daily was commenced. The following day, the patient became febrile, developed abnormal liver function and pancytopenia. Over the next week, progressive pancytopenia continued, with a nadir in her counts day 12 post-procedure: haemoglobin 81g/L, white cell count $0.6 \times 10^9/L$ and platelets $29 \times 10^9/L$. In addition, elevated ferritin (21980 ug/L, $N < 200$), triglycerides (5.1 g/L, $N < 2.0$) and LDH (438 U/L, $N < 200$) were reported.

She developed mouth ulcers (which subsequently cultured HSV1). *Herpesvirus* PCR on blood was positive however subtyping was unsuccessful.

Bone marrow biopsy demonstrated marked haemophagocytosis, consistent with HLH. Treatment with dexamethasone, G-CSF, IVIG and cyclosporine was initiated. Over the next fortnight neutrophil and platelet counts returned to normal. One month later haemoglobin and ferritin had also normalised.

Conclusion

While HLH has been noted to occur following *herpesvirus* infection and autoimmune disease, the temporal relationship of this episode to PCI and clopidogrel exposure in a previously stable patient raises the possibility of causality. Clopidogrel is associated with fever, abnormal LFTs, pancytopenia and hypersensitivity syndrome, however haemophagocytosis has not been previously reported. Two other cases of HLH following cardiac surgery have been reported. Whether this association is causal or purely coincidental remains to be determined.

No conflict of interest to disclose

A309

P094

The Haemophagocytic Syndrome – Recent Experience With Diverse Aetiologies

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The haemophagocytic syndrome is a rare and life-threatening condition characterised by excessive cytokine driven activation of histiocytes and T-lymphocytes in various organs. The aetiologies of this condition are diverse, including primary or familial forms of disease in addition to secondary haemophagocytic syndromes associated with infection, autoimmune disorders or malignancy. Given the rarity of the disorder and often non specific manifestations, diagnosis can be difficult. Clinical and laboratory criteria for diagnosis are defined, with histologic confirmation of tissue haemophagocytosis being critical. Treatment involves the use of immunosuppressive therapies to interrupt underlying cytokine dysregulation, or specific treatment directed at the underlying causative process. Allogeneic bone marrow transplantation may be indicated in familial forms of the disease.

We describe our recent experience of four patients with haemophagocytic syndrome managed at the Royal Brisbane and Womens Hospital within the last 12 months. Case 1 involves a 15 year old female with a true malignant histiocytosis with associated haemophagocytosis, whose disease was refractory to escalating chemotherapeutic and immunosuppressive therapies, resulting in a rapidly fatal outcome. Case 2 describes a 38 year old female with panniculitis like T-cell lymphoma who developed fulminant hepatic failure associated with haemophagocytic syndrome. Her disease has completely responded to a combination of alkylator therapy, dexamethasone, and cyclosporine. Case 3 is a 20 year old male presenting with an advanced mediastinal non-seminomatous germ cell tumour with severe pancytopenia due to haemophagocytosis, which has been partially responsive to BEP chemotherapy. The final case involves a 22 year old female with non-familial recurrent haemophagocytic lymphohistiocytosis complicated by recurrent life threatening infections who has undergone unrelated allogeneic bone marrow transplantation.

No conflict of interest to disclose

A310

P095**Complicated Haemolytic Anaemia in an Infant**Dorothy Stern¹, **Susan MacCallum**¹, Richard Cohn²

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Aim

To describe a complicated case of congenital haemolytic anaemia highlighting the need to consider dual aetiologies.

Background

Haemolytic anaemia in a newborn may result from congenital or acquired causes. Causes of haemolysis may be obscured by combinations of such events. Anti-B is a well known cause of neonatal haemolysis generally resolving in the first month of life. Phosphoglycerate kinase (PGK) deficiency is a rare inherited enzymopathy causing haemolysis with or without mental retardation.

Case study

Baby P (male) was born at 38 weeks. There was a maternal history of one hydropic male baby followed by two miscarriages. At delivery widespread petechiae, cardiomegaly and hepatosplenomegaly were present. Initial blood count showed haemoglobin 66g/L, reticulocytes 493x 10⁹/L, white cell count 19.3 x10⁹/L, platelets 40x10⁹/L. The blood film showed polychromasia, nucleated red cells but no spherocytosis. Baby P's blood group was B Rh(D) positive, direct antiglobulin test (DAT) negative, bilirubin 266µmol/L (0-85). The maternal group was O Rh(D) positive with a markedly elevated IgG anti-B titre of 32768. Haemolysis persisted with hyperbilirubinaemia. Baby P required frequent transfusions. Further investigations included several negative DATs and a non-diagnostic bone marrow biopsy. At three months of age he fulfilled the diagnostic criteria for Juvenile Myelomonocytic Leukaemia (JMML). By six months the JMML picture had begun to resolve spontaneously. At ten months enzyme studies showed PGK level of 16IU/gHb (195-275). Further testing revealed a new PGK gene mutation.

Conclusion

The possible causes of haemolysis in this child were anti-B and PGK deficiency. Diagnosis was complicated by the negative DATs, the JMML picture and the family history. This case illustrates that the investigation of haemolysis in a seriously ill infant is not always simple and rare causes may need to be considered.

No conflict of interest to disclose

A311

P096

Partial Clinical But Limited Laboratory Response to Prolonged Dasatinib Therapy in Systemic Mastocytosis. A Case ReportDuncan Purtill¹, Julian Cooney¹, Raja Sinniah², Paul Cannell¹¹ Department of Haematology, Royal Perth Hospital, Perth, WA, Australia² Department of Anatomical Pathology, Royal Perth Hospital, WA, Australia**Aim**

Systemic mastocytosis (SM) is associated with the D816V mutation on the proto-oncogene c-KIT. Dasatinib (BMS-354825) has been found to have significant in vitro activity against the KIT^{D816V} tyrosine kinase. It is now being trialled in patients with SM. We describe a single patient treated with dasatinib at our institution for twelve months.

Method

Dasatinib was obtained from Bristol Myers Squibb on compassionate grounds. Progress was assessed by regular clinical review, serial bone marrow samples, blood samples and sequential skin photographs.

Result

A 20 year old Aboriginal female presented with a widespread pruritic skin rash, anorexia, diarrhoea and weight loss. Skin and bone marrow biopsy demonstrated systemic mastocytosis. Molecular testing revealed the D816V mutation. Mast cell tryptase was 33.4mcg/L.

Treatment with dasatinib 50mg daily commenced as initial therapy, with steroid and anti-histamine 'pre-medication'. Initiation of treatment was associated with moderate self-limiting exacerbation of skin rash, pruritus and nausea. Dasatinib dose was increased to 50mg twice daily at one month.

The patient reported significant improvement in pruritis, although this was minor on clinical assessment. The weight remained stable. Sequential bone marrow samples showed a small reduction in mast cell infiltrate. The serum tryptase level remained stable. After ten months on dasatinib, the patient reported nausea requiring regular antiemetics. The patient suffered no pleural effusion or cytopenia. Dasatinib therapy ceased at twelve months because of patient preference to pursue traditional remedies.

Conclusion

The use of dasatinib for this uncommon condition appears safe. A transient flare of disease occurred with commencement of treatment and nausea was problematic after prolonged treatment. As has been previously documented, dasatinib may have a limited role in treating SM. In this case, symptoms from cutaneous disease improved on dasatinib. Other parameters, including mast cell tryptase and bone marrow histology, did not show response.

No conflict of interest to disclose

A312

P097

Successful Rituximab Therapy in the Treatment of Refractory Cold Haemagglutinin Disease

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Background

Cold agglutinin disease (CAD)/cryoglobulinaemia is understood to be a B-cell disorder usually resistant to treatment with immunosuppressants including corticosteroids, immunoglobulin and alkylating agents, with splenectomy employed, usually ineffectively, as a subsequent line therapy.

We describe two patients with resistant and refractory severe CAD (nadir Hb of 52 and 43 g/L) and one patient with chronic refractory moderate CAD (nadir Hb 90 g/L). All patients presented to the Launceston General Hospital, Tasmania with CAD associated severe haemolytic anaemia with high LDH and bilirubin, and positive direct antiglobulin test and undetectable haptoglobin, while CAD-titre was ranging between 1:256 to > 1:2048. Bone marrow examination in all patients showed marked erythroid hyperplasia, consistent with haemolysis, with no evidence of infiltrative marrow pathology or other haematological disorders. Initial therapy with either Prednisolone in all patients, or combined with immunosuppressive chemotherapy (Vincristine, Cyclophosphamide, Imuran) failed to control haemolysis. Subsequent treatment with Rituximab 375mg/m² IV weekly for four doses in two cases, and extended to 8 doses in one patient resulted in a significant improvement in both CAD and haemolysis. Splenectomy was not performed in any of the cases. All patients showed continuous remission of the disease after a median follow up of 24 months (range 14 to 26) with a current Hb range between 124-145 g/L. Rituximab (Mabthera; Roche, Basel, Switzerland) has demonstrated activity in the treatment of B cell lymphocyte disorders. This antibody, which binds specifically to the CD20 antigen expressed by B lymphocytes, can deplete B cells through complement- and antibody-dependent cellular cytotoxicity. Therefore, Rituximab is presumably active in immunoglobulin-mediated diseases of B lymphocytes, such as CAD.

Conclusion

Rituximab played a significant role in the successful treatment and long-term control of refractory CAD, avoiding the need to consider the uncertain benefit and the morbidity of splenectomy in this cohort of patients.

The authors confirm that there is no conflict of interest in relation to this clinical study

A313

P098

Prospective Study of Abbreviated Course Rituximab for Autoimmune Disease Refractory to Standard Immunosuppressive Therapy – An Interim ReportSZ He¹, D Ritchie¹, K Nicholls¹, T Day¹, Tuckfield AM¹, Kuss B², Matoga KE¹ and Grigg AP¹¹The Royal Melbourne Hospital, Victoria, Australia. ²Flinders Medical Centre, South Australia, Australia**Background**

Profound B cell depletion by rituximab has led to its use for antibody-mediated autoimmune diseases (AID) adopting standard anti-lymphoma dosing (375mg/m² weekly ×4). There are minimal data, however, on the efficacy of abbreviated schedules which may be more cost effective.

Aim

To investigate prospectively the efficacy of abbreviated dose rituximab in a variety of AID refractory or intolerant to conventional immunosuppression (IS).

Methods

All eligible adult patients with AID received one dose of rituximab (375mg/m²) as part of salvage therapy. A repeat dose was indicated if no (NR) or minimal response (MR) was seen 4-8 weeks after one infusion, or relapse after achieving complete (CR) or partial response (PR). Follow-up (FU) was 36 months and disease-specific autoantibodies, lymphocyte subsets and serum rituximab levels were serially analysed. Concomitant IS was tapered at physician's discretion after two weeks according to response using standard criteria.

Results

29 evaluable patients (age 22-83 years) were enrolled from March 2007 to July 2008 with 66% receiving two doses. Median FU was 24 weeks (6-45). 17 were treated for haematological conditions predominantly including immune thrombocytopenia (ITP), thrombotic thrombocytopenic purpura (TTP) and autoimmune haemolysis. Responses (CR/PR/MR) were seen in 11/1/1 in this subgroup. 7 CRs occurred after a single infusion and persisted for a median of 33 weeks (range 4+ to 42+) with one late relapse. One patient died from refractory TTP. Non-haematological conditions (n=12) consisted of mostly motor neuropathy, lupus and vasculitis with responses (CR/PR/MR) seen in 1/7/1 patient(s). In the whole study population, no grade 2-4 toxicity was observed. Following a single infusion, peripheral blood B cells were undetectable from week 2 in all patients and the earliest recovery ($\geq 0.05 \times 10^9/L$) was observed at week 24. Serum rituximab levels are in progress and will be presented.

Conclusion

The preliminary data suggest abbreviated rituximab is safe and effective in refractory AID with apparent better efficacy in haematological conditions.

This research was supported by Roche. The company had no role in analysing the data or preparing the abstract

A314

P099

Anaemia in Heart Failure: A Prospective Evaluation of Clinical Outcome in a Community Population

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Introduction

Although anaemia is well recognised to be associated with an adverse prognosis in patients with congestive heart failure (HF), the cause of the anaemia in HF and its relationship to non-cardiac as well as cardiac complications needs to be better defined, particularly in a general community population.

Methods

We followed the clinical course of 959 patients hospitalised with HF in 2004-2005 in Northern Sydney, Australia. Clinical data were collected prospectively.

Results

38% (n=369) of the patients had anaemia (Hb<120g/L). A normochromic normocytic anaemia was present in 87.8%. Of the 2/3 of patients who had more formal haematinic studies, 15.4% had a confirmed haematinic deficiency. Anaemic and non-anaemic patients were similar in age (79 vs 77 years), however anaemic patients were more likely to have elevated creatinine (48 vs 29%, p<0.001), hyponatremia (20 vs 15%, p=0.05), and LVEF >40% (49 vs 39%, p=0.004), and less likely to receive ACE inhibitors (72 vs 78%, p=0.04). Patients with anaemia had higher 12 month HF readmission rates (22.4 vs 15.7%, p=0.01) and tended to have more multiple (≥2) HF readmissions (6.3 vs 3.9%, p=0.11). Anaemic patients also had more multiple non-HF readmissions (12.4 vs 6.3%, p=0.001). The 12 month mortality was also higher for the anaemic patients (16.4% versus 10.5%, p=0.01).

Conclusion

Anaemia is common (38%) in community patients hospitalised with HF, and is associated with increased HF and non-HF readmissions, as well as increased mortality. Although most patients had normal red cell parameters, a haematinic deficiency was identified in 15.4% of patients, and is important to exclude. In conclusion, anaemia is a common, multifactorial, but potentially treatable cause of adverse cardiac and non-cardiac outcomes in patients with HF.

No conflict of interest to disclose

A315

P100

Immunotactoid Glomerulopathy and Nephrotic Syndrome Complicating Asymptomatic Chronic Lymphocytic Leukaemia

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We report the case of a 39-year-old carpenter with Rai stage 0 chronic lymphocytic leukaemia (CLL), not requiring cytoreductive treatment for four years, who developed severe peripheral oedema four years from the time of diagnosis. Despite stable peripheral blood lymphocytosis he required monthly intravenous immunoglobulin infusion for recurrent respiratory infections associated with hypogammaglobulinaemia. Peripheral oedema was exacerbated by the infusions and was associated with hypoalbuminaemia and nephrotic-range proteinuria. A renal biopsy was performed and demonstrated an immunotactoid glomerulopathy on electron microscopy, causing a clinico-pathological picture of mesangiocapillary glomerulonephritis. Immunotactoid glomerulopathy is an uncommon entity which has a recognised association with B cell lymphoproliferative disorders and dysproteinaemias. The pathogenetic mechanism is uncertain. In some case series, improvement in the glomerulonephritis is seen with treatment of CLL. Treatments described are predominantly alkylator based. Our patient's response to fludarabine, cyclophosphamide and rituximab is described.

No conflict of interest to disclose

A316

P101

Rituximab as a Novel Therapeutic Option for Orbital Benign Lymphoid Hyperplasia

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Introduction

Ocular adnexal lymphoid tumours include a number of histological entities including benign diffuse and follicular lymphoid hyperplasia, and malignant lymphoma. We report the first case of bilateral ocular benign lymphoid hyperplasia successfully treated with Rituximab monotherapy.

Method

Review of clinical, laboratory and radiological investigations was performed.

Results

A 60 year old lady with an initial presentation of left supra-orbital mass (in 2004) was diagnosed on tissue biopsy to have benign lymphoid hyperplasia of the lacrimal gland and surrounding tissues. Flow cytometry and molecular studies showed no evidence of clonality. MRI showed extensive lymphangiomatic infiltrates in retro bulbar and peri-orbital soft tissues bilaterally as well as extra-ocular facial and oral infiltrates. Repeat biopsy from a palatal lesion in 2007 showed similar histological features with negative flow cytometry; this time, clonal rearrangement of the immunoglobulin heavy chain (IgH) gene was demonstrated. There was no evidence of lymphoma elsewhere on radiology or bone marrow biopsy. She was treated with 8 doses of Rituximab (375 mg/m²) at weekly intervals. A good partial response was noted with clinical improvement in proptosis, and confirmed on objective ophthalmologic measurements including exophthalmometry and visual field assessment. MRI showed complete resolution of extra-ocular infiltrates and significant reduction in size of orbital infiltrates.

Conclusion

Rituximab monotherapy may be a safe and effective treatment for ocular adnexal lymphoid tumours.

There is no conflict of interest to disclose

A317

P102

Rituximab Therapy In Patients with Extra Nodular Manifestations of Chronic Lymphocytic LeukaemiaRaj Ramakrishna¹, Kiran Sarathy²¹ Southern Sydney Haematology & Graduate Medical School, University of Wollongong. ² Southern Sydney Haematology

Chronic lymphocytic leukaemia (CLL) is a common adult leukaemia, occurring in patients over 50 years of age with median age at diagnosis of around 65 years. While it remains an incurable disease, its indolent nature results in a varied prognosis with a median survival of greater than 10 years reported in early stage disease.

Treatment is typically initiated when patients become symptomatic including alkylating agents (chlorambucil or cyclophosphamide) but combination chemotherapy with vincristine, prednisone or fludarabine may be required in some patients. Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown significant activity in a variety of B-cell lymphoproliferative disorders. The CD20 antigen is also found on B-cells in CLL and rituximab therapy has shown clinical benefit in these patients.

Extra nodular infiltration by small lymphocytes in patients with CLL is an uncommon manifestation. There is limited data on specific therapeutic strategies for treating these patients. The following case reports highlight the usefulness of rituximab in CLL patients with extranodular infiltration, with minimal additional use of chemotherapy.

We present seven patients with various extranodular manifestations including 3 patients with cutaneous lesions with one of them showing amyloid deposits; 2 patients with renal involvement including minimal change glomerulonephritis (GN) and mesangial GN; 2 patients with pulmonary nodular infiltration. All these patients received a short course of chlorambucil therapy followed by rituximab therapy with significant improvement. The therapy consisted of weekly infusions at 375 mg/m² followed by monthly infusions for 4 months. Some of the patients have required maintenance rituximab therapy. Follow up period range: 2-8 years (median 4 years). 5 patients are alive and well and 2 patients died of unrelated causes. The surviving patients have not required any additional therapy to treat CLL or extranodular manifestations. Hence, rituximab can be used as a single agent therapy in these patients.

No conflict of interest to disclose

A318

P103

Autoimmune Neutropenia in Chronic Lymphocytic Leukaemia

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Aim

Autoimmune Neutropenia (AIN) is a rare complication of Chronic Lymphocytic Leukaemia (CLL), in contrast to autoimmune haemolysis and thrombocytopenia. There is little data in the literature and diagnosis may be confounded by many factors. We identified and analysed a small patient series to investigate the issues involved.

Methods

We reviewed the clinical records and pathology results of 4 patients seen at this institution with apparent AIN complicating their CLL.

Results

The 4 patients had profound neutropenia in the peripheral blood that was markedly disproportionate to changes in the haemoglobin or platelet counts, and with no other identified cause. All were male with a mean age of 58 (range 54-69) years. Anti-granulocyte antibodies were negative in 2 of 4 patients tested. None had received rituximab prior to the diagnosis of AIN, nor recent myelosuppressive chemotherapy. 3 had disease progression, and 2 patients have subsequently died, both due to their progressive CLL. All patients appeared to respond to combinations of G-CSF, intravenous immunoglobulin and steroids (all 4 patients) and vincristine (1 patient). All had coexistent hypogammaglobulinaemia, exacerbating the risk of infection.

Conclusion

AIN is a difficult diagnosis in CLL, particularly after any therapy, and likely to become more so with increasing use of rituximab. Its incidence is possibly underestimated and complicated by the lack of a reliable and readily accessible diagnostic test. When present, it appears to respond to steroids, intravenous immunoglobulin and cytokine (G-CSF) therapy. Most patients in this series have had subsequent progression of their CLL.

No conflict of interest to disclose

A319

P104

Correlation of IgVH Mutation Frequency with Zap 70 and CD38 expression in a Cohort of South Australian CLL PatientsRachel Hall¹, Sheree Bailey², Peter Macardle², Bryone Kuss¹¹Department of Haematology & Genetic Pathology & ²Department of Immunology, Flinders Medical Centre, South Australia**Aim**

The degree of mutation frequency (MF) of the IgVH gene is a good determinant of prognosis in CLL patients. Zap70 and CD38 expression have also been reported as surrogate markers for CLL prognosis, however disparate results have been widely reported. This maybe, in part, due to selection of patients presenting to tertiary medical institutions. Therefore, the aims of this study were to examine IgVH Somatic Hyper-Mutation (SHM) status and correlate Zap70 and CD38 expression in a cohort of patients representing CLL in the normal population.

Method

76 CLL patients were examined for IgVH SHM status, Zap70 and CD38 expression. The IgVH gene of each patient was amplified using standard PCR, then sequenced, and the MF determined using the IMGT database. Zap70 (2F3.2-FITC UpState) and CD38 (AT13/5-A647 Serotec) were examined by Flow Cytometry with a calibrated FACS Canto (BD Bioscience). Zap 70 expression was calculated in a ratiometric analysis of MFI of CD19⁺/CD5⁺ cells/MFI of CD5⁺/CD19⁻ cells. CD38 expression was reported as MFI. Statistical analysis was by correlation coefficient.

Results

Zap70 expression and IgVH mutation frequency showed low Zap70 expression (25% percentile) correlating with high MF. High Zap70 expression (75% percentile) correlated with low MF. CD38 expression was inversely correlated with IgVH SHM status ($p < 0.05$). A direct comparison between Zap70 and CD38 showed a correlation between low Zap70 and low CD38, however no trend was evident between high Zap70 and high CD38.

Conclusion

In this study there was limited correlation between Zap70 expression and IgVH SHM frequency with a relationship only evident at the upper and lower 25% percentiles, with the majority of patients falling into a grey area. CD38 expression has been proposed as an independent prognosticator in CLL and showed an inverse relationship with IgVH SHM frequency.

No conflict of interest to disclose

A320

P105**High Frequency of Homozygous Deletion on 13q Associated with Deletion of p53 in CLL in a Cohort of 65 Consecutive Cases**

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Background

The most common genetic aberrations that determine prognosis in chronic lymphocytic leukemia (CLL) include deletions of regions within chromosomes 13 [del(13q)], 11 [del(11q)] and 17 [del(17p)] with increasing risk respectively. Detection of these aberrations by routine karyotyping has been limited due to the low proliferative rate of the malignant B cells. Fluorescent in-situ Hybridization (FISH) is widely used as it can be applied to either blood or bone marrow in CLL and does not require metaphase spreads.

Methods

65 consecutive CLL cases seen at Royal North Shore Hospital were evaluated by both karyotyping and FISH using the Vysis CLL panel of probes.

Results

57% of results were abnormal by FISH vs 17% by karyotyping. 26 cases (40%) showed del(13q); 7 of which had homozygous interstitial deletions in a proportion of the cells analysed. Interestingly, 5 of these 7 cases showed deletion of 17p, compared with 4 of the 19 cases in which only heterozygous 13q loss was detected. 2 cases with del(17p) were shown to have evolved from heterozygous to homozygous loss of 13q. The karyotype was normal in the 3 cases where metaphase spreads were established. No correlation with disease stage or CD38 expression was seen.

Conclusion

Genomic instability conferred by del(17p) may account for the increased incidence of homozygous 13q loss in this series, however this was not reflected in the karyotypic analyses performed. This high incidence of del(17p) in homozygous del(13q) cases has not been previously documented. Clinical follow-up is ongoing with this patient cohort.

No conflicts of interest to disclose

A321

P106

ATM Inactivation as a Consequence of *TP53* Mutation in B-CLLGiles Best^{1,2}, Anne Gardiner¹, **Stephen Mulligan**² and David Oscier¹¹ *Department of Haematology, Royal Bournemouth Hospital, Bournemouth, UK*² *Department of Haematology, Royal North Shore Hospital, Sydney, NSW, Australia***Background**

ATM phosphorylation of TP53 is an integral step in limiting cell cycle progression and initiating apoptosis following double stranded DNA damage. Recent evidence suggests that certain 'gain-of-function' *TP53* mutations may interfere with ATM activation.

Methods

Using a novel flow cytometric assay we studied the response of B-CLL cells to DNA damage. We exposed patient samples to etoposide and measured the up-regulation of TP53 and p21 and increase in ATM phosphorylation on serine 1981 (ATM^{S1981}). ATM and TP53-related dysfunction were distinguished by incubation with etoposide and the Mdm-2 inhibitor nutlin-3a. In a separate study we demonstrate that ATM or TP53-related dysfunction is consistent with detection of mutations in the corresponding gene.

Results

We analyzed samples from 93 patients using our functional assay. We identified 54 cases as functionally normal, with 20 ATM dysfunctional and 19 TP53 dysfunctional cases. The mean percentage increase above baseline in ATM^{S1981} in the functionally normal cases was 34%. As expected 19 of the 20 (95%) cases with ATM dysfunction had low or completely attenuated ATM^{S1981} with a mean increase of only 6%. Surprisingly, 9 of the 19 (47%) cases with TP53 dysfunction had low ATM^{S1981} with a mean increase of only 15%. This was significantly lower than observed in the functionally normal cases ($p < 0.005$).

Conclusions

Our data are the first evidence of an ATM feedback mechanism in B-CLL. Studies into the mechanism underlying the data, including whether this phenomenon is dependent on the site of *TP53* mutation, are on-going. Clinically FISH stratifies prognosis in CLL with deletion of 11q (ATM) and 17p (TP53) associated with worse prognosis. We suggest that this data represents further rationale for the worse outcome of patients with 17p compared to 11q loss.

No conflicts of interest to disclose

A322

P107

8p11 FGFR1 Gene Rearranged Myeloproliferative Disorder Treated with the Investigational Tyrosine Kinase Inhibitor (TKI) Midostaurin

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Introduction

Rearrangement of FGFR1, located at 8p11-p12, with various fusion partner genes is associated with a rare stem cell myeloproliferative disorder. It usually presents in chronic phase with marked eosinophilia but progresses rapidly with a very poor prognosis to acute leukaemic transformation of myeloid, T or B lymphoblastic lineage. It is resistant to imatinib but other TKIs may be candidate therapies. We describe a case who received treatment with PKC412 (midostaurin), an investigational oral multi-TKI with activity against FLT3, PDGFRA, PKC alpha, beta and gamma, FGFR1, FGFR3 and KIT receptor tyrosine kinases, currently being studied in a phase 3 trial in FLT3-AML.

Case Report

A 63 year old female presented incidentally with prominent neutrophilia and eosinophilia. She later developed constitutional symptoms, nausea, diarrhoea, cervical lymphadenopathy, hepatosplenomegaly, low level circulating blasts, and deteriorating anaemia and thrombocytopenia. Bone marrow biopsy showed marked myeloid hyperplasia with eosinophilia with blasts < 5%. Cytogenetics revealed rare t(8;9)(p12;q33) and FGFR1 rearrangement was confirmed by BAC-FISH. The patient was considered ineligible for stem cell transplantation and after initial hydroxyurea the patient was commenced on single agent midostaurin. This produced beneficial early clinical and biological effects (improved bone pain, stabilized weight, decreased hepatosplenomegaly, reduced white cell count, and resolution of circulating peripheral blood blasts) but after 5 months of therapy the patient developed abnormal LFTs and increasing anaemia without other clinical features of disease progression and the drug was suspended. Within one week the patient showed obvious features of disease progression and CT scan showed periportal and other widespread lymphadenopathy suggesting that the initial LFT disturbance was related to disease progression.

Conclusion

FGFR1 rearranged MPD is a rare poor prognosis disease without established therapy. Midostaurin shows evidence of efficacy and in combination with other strategies may further control initial disease in patients eligible for consideration of stem cell transplantation.

No conflict of interest to disclose

A323

P108

The Intracellular Transport Mechanisms of Nilotinib (AMN107) Are Different to Imatinib, and May Indicate a Strong Association of This Drug with ATP Dependent EffluxLaura Eadie¹, Amity Frede¹, Timothy Hughes^{1,2} and Deborah White^{1,2}

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Aim

We previously demonstrated transport of nilotinib is different to imatinib, however transport kinetics remain unclear. Knowledge of proteins and processes involved is essential to the understanding of drug:drug interactions. Hence our aim was to investigate intracellular uptake and retention (IUR) kinetics of nilotinib.

Method

IUR assays were performed as previously using ¹⁴C-nilotinib and imatinib. Time and temperature dependence were investigated in cell lines, and CML mononuclear cells.

Results

After 15 mins, there was no significant difference in the IUR of nilotinib between 4°C and 37°C ($p=0.649$), however after 2 hours the IUR at 37°C was significantly less than that at 4°C ($p=0.003$). In contrast the IUR of imatinib was significantly higher at 37°C than 4°C ($p=0.003$ and 0.018) at both timepoints. This suggests the uptake (first 15 mins) of nilotinib is efficient and predominantly passive, however a temperature dependent (37°C-ATP related) efflux mechanism subsequently dominates, resulting in reduction in IUR at 37°C, but not 4°C. This contrasts with the OCT-1-dependent active transport of imatinib. Investigation of nilotinib efflux using ABCG2 and ABCB1 over-expressing cell lines, revealed no difference ($p>0.05$) in nilotinib IUR between these and parental lines. Treatment with known inhibitors of ABCB1 and ABCG2 did not reverse efflux ($p>0.05$). Investigating the effect in primary cells ($n=10$), of proton pump inhibitors known to inhibit ATP dependent efflux, revealed a significant increase in IUR at 37°C after 2 hours ($p<0.002$) indicating reversal of efflux, not seen with imatinib.

Conclusion

While on initial exposure nilotinib is primarily taken into the cells in an ATP independent manner, longer exposure results in altered transport kinetics with ATP dependent efflux predominating. Other efflux transporters besides ABCB1 and ABCG2 are likely involved. The significant impact of drugs which inhibit ATP related efflux, may be of clinical relevance in the nilotinib setting, in contrast to the findings with imatinib.

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A324

P109

Preliminary Testing of a Rapid Quantitative PCR for BCR-ABL Detection: The Cepheid GeneXpert BCR-ABL Monitor System

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Introduction

Real-time quantitative reverse-transcriptase PCR (RQ-PCR) detection of BCR-ABL mRNA is the gold standard for monitoring response to treatment of CML patients. The novel Cepheid GeneXpert BCR-ABL Monitor (GX) is a self-contained cartridge-based system, which utilises pre-loaded software on a personal computer to perform RQ-PCR detection of p210 transcripts in peripheral blood, in less than 2.5 hours.

Aim

To undertake a preliminary evaluation of the GX, within a routine haematological cytogenetics (non-molecular) laboratory.

Method

54 peripheral blood (EDTA) samples (1 newly diagnosed CML; 53 follow-up CMLs) were tested according to procedures and conditions provided with the GX kits. The samples were also tested in parallel at one of 4 reference sites routinely performing BCR-ABL RQ-PCR testing.

Results QUALITATIVE PCR

	Reference NEG	Reference POS
GX NEG	8	2
GX POS	0	1

QUANTITATIVE PCR

	Reference NEG	Reference POS
GX NEG	17	3
GX POS	0	23

- Sensitivity = 83% (24/29)

5 false negatives: 2 were p190 transcripts by qualitative PCR (not detected by GX)
2 samples were stored overnight before testing – 0.006% & 0.03% levels detected by reference lab. 5th false negative found to have a transcript level of 0.001% by reference lab (cut-off level of detection by the GX).

- Specificity = 100%
- As expected due to different methodologies, absolute transcript levels detected by the GX were different to those of the reference laboratory, but comparable when normalised to the same baseline.
- 4 Imatinib patients tested serially over 6 months showed consistent results with reference testing and GX. 3 patients showed a 1-log reduction in BCR-ABL transcripts over 6 months; 1 patient showed complete lack of molecular response.

Conclusion

Preliminary testing of the GX has yielded very promising results, prompting us to continue quantitative precision testing of the system. However discrepancies between results for samples with low-level transcript numbers require further investigation. Lack of p190 and p230 transcript detection is a disadvantage but is likely to be addressed with the second generation of instrument.

No conflict of interest to disclose

A325

P110

DNA-based Monitoring of Minimal Residual Disease (MRD) in Chronic Myeloid Leukaemia (CML)

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Aim

To develop a DNA-based method for monitoring MRD in CML and compare it to the conventional RNA-based method.

Methods

For DNA-based monitoring, the previously-determined sequence of the *BCR-ABL* breakpoint was used to synthesise PCR primers and MRD was quantified in a three round nested PCR. For RNA-based monitoring, conventional reverse transcriptase PCR (RT-PCR) was used.

Results

Preliminary results using mixing experiments showed that DNA-PCR could accurately quantify MRD down to 10^{-6} without confounding by nonspecificity. To date, 26 samples from 20 patients have been studied by both methods during treatment. In 13 patients MRD down to approximately 10^{-4} was measured by both methods, the correlation coefficient between them being 0.85; in 5 patients disease could not be detected by either method; in 7 patients disease was quantified only by DNA-PCR; and in one patient disease was quantified only by RT-PCR. The level of detection of RT-PCR was approximately 10^{-4} whereas that of DNA-PCR was approximately 10^{-6} . For 19 samples MRD was quantified by DNA-PCR in 2 independent experiments. The SD of these replicates was 0.38 log units. Some difficulty in primer design and/or some non-specific amplification in the PCR were observed in three patients in whom *Alu* sequences flanked the *BCR-ABL* breakpoint.

Conclusions

DNA-based monitoring of MRD in CML has several advantages compared to RNA-based monitoring. Collection, processing and transport of samples is simpler and more robust, reverse transcription is not required and quantification is more sensitive. The disadvantages are that extra resources are required for the initial steps of isolation and sequencing of the breakpoint and synthesis of patient-specific primers. However the cost of these initial steps may be able to be amortised over the multiple tests which are performed during monitoring of treatment.

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A326

P111

Heterogeneity of Genomic BCR-ABL Fusion Sequences in Chronic Myeloid Leukaemia Patients

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Translocation of ABL1 to fuse with BCR is an initiating event in chronic myeloid leukaemia (CML), yet our understanding of this process is limited. It is technically difficult to sequence the genomic breakpoints of CML patients and only small series have been published.

Aim

To examine the distribution and nucleotide structure of BCR-ABL breakpoints and recombination events.

Methods

DNA samples were obtained from 37 chronic phase CML patients and 5 BCR-ABL cell lines, all expressing b2a2 or b3a2 BCR-ABL mRNA. PCR products containing the BCR-ABL fusion were identified by long PCR with a single forward primer in BCR and multiple reverse primers distributed from ABL1 exon 1b to exon 2. In a subset of 8 samples primers were used to amplify the predicted reciprocal ABL-BCR fusion on der(9).

Results

BCR breakpoints occurred in intron 13, exon 14, and intron 14 (3kb). ABL1 breakpoints occurred in intronic regions from upstream of exon 1b to intron 1a (140kb). No obvious clustering of breakpoints was identified. 62% of ABL1 breaks were within genomic repeat regions, versus 14% of BCR breaks. 62% of BCR-ABL fusions involved microhomology with 1-5 bases of identity in the BCR and ABL1 ends. The ABL-BCR reciprocal translocation was 'unbalanced' at a nucleotide level in 3/8 with duplication of normal ABL1 and/or BCR sequences adjacent to the breakpoint (median length 100bp).

Conclusions

Deletion and microhomology at the recombination site is evidence of non-homologous end-joining with 'micro'single strand annealing. Duplication of BCR or ABL1 sequences in both BCR-ABL and ABL-BCR was common in the small number analysed so far. Duplication might arise from staggered DNA breakpoints in one gene, with duplication of the sequence between the two breaks. The molecular anatomy of BCR-ABL fusions is heterogeneous. The biological and prognostic significance of this diversity warrants further investigation.

No conflict of interest to disclose

A327

P112

Initial Experience of Alemtuzumab Used as Salvage Therapy for Etanercept-refractory Acute GVHD

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Aims

To review the outcome of etanercept-refractory acute graft versus host disease (GVHD) salvaged with alemtuzumab-based therapy at our institution.

Methods

Retrospective review of post-allogeneic stem cell transplant patients treated with alemtuzumab for refractory GVHD. Alemtuzumab was used in all cases as 3rd line therapy after failing initial therapy with methylprednisolone then 2nd line therapy with ATGAM and etanercept +/- mycophenolate. Alemtuzumab dose was 10mg subcutaneously for 5 consecutive days (BBMT 2008; 14: 10). Response was assessed at 28 days following commencement of alemtuzumab.

Results

To date, 4 patients have received alemtuzumab for refractory acute GVHD. All 4 patients had grade IV GVHD, with the predominant organ involved the gastrointestinal tract in 3 and skin in 1. At 28 days post-alemtuzumab, 2/4 patients achieved complete resolution of their GVHD, including 1 patient with gastrointestinal GVHD and the 1 patient with skin GVHD. With short follow-up (<6 months), 2/4 patients have died, including 1 responding patient and 1 non-responder. Causes of death included sepsis / invasive fungal infection (n=1) and progressive GVHD (n=1). Other significant infections post-alemtuzumab have included multifocal bacterial pneumonia complicated by abscess formation (n=1) and CMV reactivation (n=2).

Conclusions

Alemtuzumab appears to induce promising responses in GVHD refractory to multiple previous lines of therapy. Infectious complications appear significant, though not out of keeping with that expected in such a heavily immunosuppressed patient population. Further study of alemtuzumab as therapy of refractory GVHD is warranted.

Authors do not know of any conflict of interest

A328

P113**Acute Graft-versus-host Disease after Liver Transplantation**

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Aim and Method

Acute Graft-versus-host Disease (GVHD) is a very rare complication of liver transplantation. Its presenting features include pancytopenia, rash and diarrhoea. It has a high mortality and optimal management is unknown. We reported two male patients from our institution who developed acute GVHD after orthotopic liver transplantation. Their clinical features, investigations, treatments and progress, with a review of literature, were presented.

Result

Patient 1, age 57, had hepatitis C complicated by hepatocellular carcinoma (HCC). He received a liver transplant from a 27 year old female cadaveric donor. Patient 2, age 65, had hepatitis B and HCC. He received a cadaveric liver transplantation from a 74 year old female.

Their operations were uneventful. Both patients developed pancytopenia, rash and diarrhoea one month post transplantation. Bone marrow biopsies showed marked hypocellularity with evidence of mixed chimerism. Skin biopsies were consistent with acute GVHD. Patient 1 was treated with methylprednisolone, cyclosporin and basiliximab and died six days later from multiorgan failure. Patient 2 was treated with methylprednisolone, cyclosporin, mycophenolate mofetil and etanercept. He recovered from acute GVHD, however aspergillus infection supervened, which responded to voriconazole and posaconazole. He is currently stable and well eight months after his liver transplant.

Conclusion

Acute GVHD post liver transplantation is a rare complication with high mortality, usually from bone marrow failure. Its presenting features may mimic common transplant complications such as drug side effects and infections. Hence a high index of suspicion is necessary. Risk factors include age, and in contrast to allogeneic bone marrow transplant (BMT), close HLA matching. The diagnosis remains clinical with appropriate histology and evidence of chimerism.

There is no evidenced based treatment at present and management is guided by experience in the more common acute GVHD seen after allogeneic BMT. There is evidence for etanercept use as first line therapy with steroids in acute GVHD following allogeneic BMT. There is no prior published literature on its use in the liver transplantation setting.

No conflict of interest to disclose

A329

P114

Infusion of Mesenchymal Stromal Cells for Later Graft-versus-host Disease after Allogeneic Haemopoietic Stem Cell Transplantation

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Aims

Infusion of mesenchymal stromal cells (MSC) for acute graft-versus-host-disease (GVHD), refractory to steroid therapy, has received increasing attention and is currently the subject of an international phase III study. Local ethics approval was obtained for a phase I study of infusion of MSC in patients not eligible for the phase III study, with the aim of this study being to demonstrate safety and efficacy of the treatment.

Methods

To date, four patients have been treated. Two patients were very late after transplantation, namely seven and eight years, both had obliterative bronchiolitis together with conjunctival GVHD and the sicca syndrome. The other two patients were five and six months post transplant and predominantly had cutaneous GVHD of extensive degree, one with hepatic GVHD and one with severe oral mucosal GVHD.

MSC were culture expanded from marrow taken from the original sibling donor or, in the case of the unrelated donor transplants, one received MSC from haploidentical family donors whilst the other received MSC from an unrelated donor. MSC were characterised by morphology and phenotype. The median dose of MSC was 1.7×10^6 MSC/kg (range 0.3 - 2.1), infused fresh or cryopreserved, twice weekly for four weeks or given as required.

Results

Two patients with obliterative bronchiolitis did not improve subjectively or objectively in relation to the obliterative bronchiolitis. One of these patients had significant improvement in his conjunctival GVHD. The other two patients with predominantly cutaneous GVHD both had complete responses. One patient required repeated intermittent infusions over a period of ten months for relapses of the cutaneous GVHD, on each occasion achieving a complete response. No adverse effects were seen from infusion of MSC.

Conclusions

Infusion of MSC can be effective in later GVHD in selected cases. With the really late cases with obliterative bronchiolitis the long term scarring associated with this condition precluded a response. Further studies in early chronic GVHD are indicated.

No conflict of interest to disclose

A330

P115**Chronic Graft-versus-host Disease and Remission Status Are Significant Factors for Survival Following HLA-matched Stem Cell Transplantation for Acute Myeloid Leukaemia****John Kwan**, Anthony Dodds, Sam Milliken, Keith Fay, Awachana Jiamsakul, David Ma, John Moore*Department of Haematology & Stem Cell Transplantation, St Vincent's Hospital, Victoria St, Sydney NSW 2010***Background and Aims**

Allogeneic stem cell transplantation (SCT) for acute myeloid leukaemia (AML) is a well-established curative treatment modality. Improving survival outcomes relies on identifying factors, which limit toxicity and maximise the graft-versus-leukaemia (GVL) effect responsible for long-term disease control. This study aimed to determine factors associated with overall and disease free survival in a continuous cohort of patients with AML who underwent HLA-matched myeloablative or non-myeloablative SCT.

Methods

Eighty-six (n=86) patients underwent HLA-matched myeloablative or non-myeloablative SCT for AML between 1996 and 2008 at this institution. Conditioning regimens included cyclophosphamide/TBI, busulphan/cyclophosphamide or fludarabine/melphalan. Cord blood transplants were excluded. Combination GVHD prophylaxis was used (cyclosporin, methotrexate, prednisone) with GVHD scored using standardised CIBMTR guidelines. Data sets were analysed using multi-variate Cox regression models, Kaplan-Meier analysis & the log-rank test.

Results

Forty-three of 86 patients (50%) have survived with a median follow-up of 33.4 months. Median age was 42 years (range 15-67). Thirty patients (35%) received unrelated transplants. Sixteen patients (19%) received non-myeloablative conditioning. The only significant factor for overall survival was patients in any remission (CR1 or greater) compared with relapsed or refractory disease (Relative Risk(RR) 2.2, p=0.01). Significant factors for disease free survival were the presence of chronic graft-versus-host disease (RR of relapse/death 0.3, p=0.04) and patients in first CR (RR 0.281, p=0.005). No other factor including age, CMV status, conditioning intensity or donor characteristics (including related/unrelated) were significant factors in multi-variate analysis.

Conclusions

This analysis confirms the importance of disease stage and chronic graft versus host disease as significant factors in survival for patients transplanted with AML. The presence of chronic GVHD could be used as a surrogate marker for the graft-versus-leukaemia effect and underlines its importance in disease control. Donor source and conditioning regimens are not significant factors in survival consistent with recently published data.

No conflict of interest to disclose

A331

P116

Detailed Profiling of Allogeneic T Cell Phenotype in HLA-Matched and -Mismatched Donor/Recipient PairsJoanne Davis¹, Paul Neeson¹, Joseph Trapani^{1,2}, **David Ritchie**¹

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Aim

Allogeneic bone marrow transplantation is commonly utilised for the definitive treatment of leukaemias and lymphomas. Graft-versus-host disease (GVHD) is the major risk of such an approach, resulting in considerable morbidity even in sibling HLA-matched donor/recipient pairs. In vitro analysis of allogeneic T cell behaviour may predict the onset of acute GVHD in bone marrow transplant (BMT) recipients.

Method

In order to define parameters for subsequent HLA-matched BMT, we utilised the mixed lymphocyte reaction (MLR) of 20 healthy HLA-mismatched responder/stimulator pairs to monitor the emergence of alloreactive effector CD4⁺ and CD8⁺ T cells by multiparameter flow cytometry, including central memory and effector T cell subsets, and CD4⁺CD25⁺CD127⁺FoxP3⁺ regulatory T cells (Tregs). Additionally, Th1/Th2 cytokine secretion profiles were measured on days 2 and 7 of MLR by cytokine bead array. Several HLA-matched siblings were tested prior to BMT to examine their alloreactive responses in comparison with HLA-mismatched MLR.

Result

Allogeneic T cells stimulated in HLA-mismatched MLR gained an effector phenotype (CD3⁺CD8⁺CD27⁺CCR7⁺CD45RA⁺perforin⁺) after 7 days, and secreted the Th1 and Th2 cytokines IL-2, IL-4, IL-5, IL-10, IL-13, IFN γ and TNF α . Upregulation of IFN γ , IL-13 and IL-2 secretion correlated with alloreactive T cell proliferation. In four HLA-matched MLR, allogeneic T cell proliferation and cytokine release was minimal, although TNF α secretion by donor T cells was elevated in comparison to HLA-mismatched MLR levels. Interestingly, Tregs were present at baseline and day 7 of both HLA-matched and -mismatched MLR.

Conclusion

Our studies have defined the emergence of alloreactive effector CD4⁺ and CD8⁺ T cells, in addition to the typical range of cytokine production in HLA-mismatched pairs. These findings have provided parameters for predicting GVHD in potential BMT donor/recipient pairs. We are currently testing further HLA-matched sibling pairs to identify the secretion of key cytokines that may influence post-transplantation complications.

No conflict of interest to disclose

A332

P117**GSTM1 and GSTT1 Gene Polymorphism in Patients with Bone Marrow Failure Syndromes in North India**

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Introduction

Bone marrow failure syndromes (BMFS) comprise of aplastic anemia and myelodysplastic syndromes (MDS). BMFS are classified as constitutional, acquired secondary and acquired primary subtypes. The definition of exact etiology is important for the obvious reasons of proper management, family screening and genetic counseling etc. Constitutional factors reportedly contribute to BMFS in nearly 1/3 of young patients. The acquired factors include drugs, chemicals, toxins, infections (mainly viral) etc. The exact frequency of multiple etiological factors, especially constitutional, is not well documented. Most of the genetic predisposition syndromes (chromosomal instability syndromes, constitutional chromosomal disorders and toxin modifying enzymes variants etc.) require specific diagnostic tests and many (like Fanconi anemia) require modified treatment strategy. Data on etiological factors is largely not available from Indian subcontinent.

Materials and methods

The PCR genotyping for GSTM1 and GSTT1 (toxin modifying enzymes variants) was undertaken in 112 aplastic anemia patients, 50 MDS patients and 106 normal healthy controls, as a part of Department of Biotechnology sponsored project.

Results

Normal GST genotype was detected in 57 aplastic anemia patients, 23 MDS patients and 88 control individuals. GSTT1 null genotype was found in 12 aplastic anemia patients, 10 MDS patients and 8 control individuals ($p>0.05$). Prevalence of GSTM1 null genotype was found to be significantly more in BMFS (26 aplastic anemia and 21 MDS patients) as compared to 8 control individuals ($p<0.01$). Composite GSTM1 and GSTT1 null genotype was found in 4 aplastic anemia patients.

Conclusions

GSTM1 null genotype most likely contributes to the causation of BMFS in our patient population.

No conflict of interest to disclose

A333

P118

Compartmentalization of Allogeneic-T cell Responses in the Bone Marrow and Spleen of Humanized NOD/SCID Mice Containing Activated Human Resident Myeloid Dendritic Cells

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The human allogeneic (allo)-T cell responses within recipient lymphoid tissues and the degree to which they are altered by activated tissue-resident dendritic cells (DC) remains unknown. This study examined human allo-T cell recruitment and the early allo-T cell responses within the bone marrow (BM) and spleen (SP) of humanized (hu)NODSCID recipients containing activated human tissue-resident myeloid (M)DC.

Human naïve allo-T cells were transferred into poly(I:C) treated or untreated huNOD/SCID recipients containing human tissue-resident DC derived from transplanted CD34⁺ cells. The activation of human tissue-resident MDC by poly(I:C) treatment, and the recruitment, proliferation and effector differentiation of allo-T cells in the BM and SP of huNOD/SCID recipients were analysed *in vivo* by flow cytometry.

Poly(I:C) treatment induced transient activation of human MDC within a maximum of 8 hours, evident in the BM as an increased proportion of MDC expressing CD86 and in the SP by MDC expressing CD86 and producing IL-12. Poly(I:C) pretreated huNOD/SCID recipients recruited allo-T cells into both the BM and SP but developed different allo-T cell responses within the BM and SP compartments. In the BM, allo-T cells underwent multiple divisions and increased numbers of IFN- γ ⁺ and TNF- α ⁺ effector cells, whereas the majority of splenic allo-T cells underwent a single division and had fewer effector allo-T cells. This experimental transplantation model demonstrates that early human allo-T cell responses are regulated by compartmentalization in the BM and secondary lymphoid tissues such as spleen; events that provide potential insights into clinical transplant reactions.

No conflict of interest to disclose

A334

P119

Mechanisms of Histone Deacetylase Inhibitor induced Thrombocytopenia

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Aims

Histone deacetylase inhibitors (HDACi) are novel anti-cancer agents which have undergone rapid clinical development in phase I/II trials. These drugs have single agent efficacy in relapsed/refractory acute myeloid leukaemia, hodgkin lymphoma, myeloma and cutaneous T-cell lymphoma. Reversible thrombocytopenia is a consistent dose limiting toxicity for this class of drugs, and does not appear to be due to a direct cytotoxic myelosuppressive effect, and may limit future combination treatment strategies. We aim to dissect the mechanisms of thrombocytopenia, to see if it may ultimately be circumvented.

Methods

More than 150 BALB/c and C57BL/6 mice, in several groups, have been dosed with daily intra-peritoneal panobinostat (LBH-589) and controls at a range of concentrations (10-20mg/kg) on weekly and alternate weekly schedules. Full blood examinations were performed daily in consecutive groups throughout treatment. Serum thrombopoetin (TPO) levels were measured by ELISA. The age profile of platelets was measured using thiazole orange (TO) to stain younger, reticulated platelets prior to quantitative flow cytometry.

Results

Both C57BL/6 and BALB/c mice treated with panobinostat have predictable, progressive reduction in platelet counts compared to controls, from the normal range of $900-1100 \times 10^9/L$ to a nadir of $200-400 \times 10^9/L$ at five days ($P < 0.001$). Further dosing only results in a plateau in levels rather than further reduction. On cessation, there is a rapid rebound thrombocytosis ($1300-1500 \times 10^9/L$) prior to normalisation over a week. Re-challenge with panobinostat results in an identical pattern of thrombocytopenia. TPO levels appeared to be unaffected by administration of panobinostat. TO staining and bone marrow are underway and will be presented.

Conclusions

Panobinostat induced thrombocytopenia is predictable, and appears to be self limiting regardless of dose. Endogenous TPO levels do not alter in response to the thrombocytopenia. Further investigations will focus on whether effects are intrinsic to effects on platelets themselves or production by megakaryocytes and pro-platelet formation.

No conflict of interest to disclose

A335

P120

Acute Myeloid Leukaemia (AML) as a Model to Dissect MicroRNA Transcription Networks During Myelopoiesis**Mark Lutherborrow**¹, Vivek Jayaswal², Adam Bryant¹, Yee Hwa Yang², David Ma¹¹*Blood Stem Cell and Cancer Research Unit, St Vincent's Hospital Applied Biomedical Research, Darlinghurst, NSW, Australia* and ²*School of Mathematics and Statistics, University of Sydney, Sydney, NSW, Australia***Aim**

To investigate the role of miRNAs in the characteristic differentiation block observed in AML using microRNA (miRNA) and mRNA microarray analysis of normal karyotype AML

Methods

28 diagnostic bone marrow samples were obtained after informed consent. Mononuclear cell fractions averaging 75% blasts were isolated and subjected to miRNA microarray analysis using the Exiqon platform. Computational mRNA targets of differentially expressed miRNAs were identified and correlated with mRNA array data published by Valk, *et al.* N Engl J Med. 2004.

Results

69 human miRNAs were found to have a significant change (< or > 1.5 fold) in AML samples when compared to normal bone marrow. Clustering of miRNA expression profile showed poor clustering of all FAB subtypes present, except, 9 miRNAs were able to segregate the M1 (n=7) and M5 (n=7) FAB subtypes. mRNAs that were differentially expressed between these two FAB subtypes were identified in the microarray database. Computational mRNA targets of the 9 miRNAs were then correlated with this data to identify putative miRNA:miRNAs pairings. Two sets of pairings were found to link with myeloid and monocytic differentiation respectively. These differentially expressed miRNAs may explain the respective monocytic and myelocytic differentiation block seen in the M5 and M1 subtypes respectively.

Conclusions

miRNAs have been shown to be extremely important in haematopoiesis and the development of cancer. We show the potential of miRNA profiling of subtypes of AML as a model to investigate haematopoiesis, particularly in the differentiation block observed in these AML subtypes.

No conflict of interest to disclose

A336

P121

Anti-IgD Antibody is a Novel Modulator of T-Bet in Human Peripheral Blood Mononuclear Cells

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Aims

The transcription factor, T-Box expressed in T-cells (T-Bet), is a regulator of T-helper 1 (Th1) immune responses, which are enhanced in several autoimmune and inflammatory conditions. Experiments with murine models have demonstrated that treatment with anti-IgD antibody induces a bias towards a Th2 response. However, the effects of anti-IgD antibody on human Th1/Th2 immunity are unknown. The aims of this pilot study were to determine:

1. effects of anti-IgD antibody treatment on T-Bet expression in human peripheral blood mononuclear cells (PBMCs)
2. expression levels of surface membrane IgD (smlgD) on PBMCs.

Methods

Peripheral blood (45 mL) was collected from 13 healthy males (n=6) and females. PBMCs, B-cells and monocytes were isolated using standard techniques. The effects of anti-IgD antibody (10 μ g/mL to 100 μ g/mL) on T-Bet were determined by RT-PCR and western blot. Expression of smlgD on each cell population was examined by dual-immuno-fluorescent labelling flow cytometry, using PE and FITC conjugated antibodies specific for cell markers and smlgD respectively.

Results

Using RT-PCR, anti-IgD had a dose-dependent modulating effect on T-Bet expression when cells were stimulated with PMA and ionomycin. Using flow cytometry and immunocytochemistry, B-cells and monocytes appeared to express IgD, or an IgD-like molecule, on the cell surface.

Conclusions

This pilot study is the first to describe the effect of anti-IgD antibody on T-Bet expression levels in human PBMCs. Results indicate a dose-dependent effect of anti-IgD on T-Bet expression. Successful control of the expression of T-Bet, and hence down-regulation of the Th1 immune response, may have important implications for future treatment of autoimmune and inflammatory conditions.

No conflict of interest to disclose

A337

P122

The Th1 Regulatory Molecule, T-Bet, is Increased *In Vitro* by Recombinant Human Osteopontin in Peripheral Blood Mononuclear CellsPamela Sheahan^{1,2}, Meilang Xue¹, Chris Jackson¹, Nghia Le¹, **Narelle Woodland²**¹*Kolling Institute of Medical Research, University of Sydney at Royal North Shore Hospital, NSW, Australia,* ²*University of Technology Sydney, NSW, Australia***Aims**

T-Box expressed in T-cells (T-Bet) is a transcription factor that has an essential regulatory role in T-helper 1 (Th1) immune responses, such as those seen in Rheumatoid Arthritis (RA). Osteopontin, a phosphoprotein, is highly expressed by fibroblast-like synoviocytes in RA synovium. While osteopontin is associated with bone resorption and tissue remodelling, its role in RA is unknown. The aim of this project was to determine whether recombinant human osteopontin (rhOPN) alters the gene expression of pro-inflammatory cytokines or molecules associated with regulatory function in unstimulated peripheral blood mononuclear cells (PBMCs) *in vitro*.

Methods

Peripheral blood (40mL) was collected from 12 healthy adults. PBMCs were isolated and cultured for 24 hr at 37°C with and without rhOPN (1ug/mL). Cytokine gene expression was determined by RT-PCR and PCR, using primers for IL-1 β , IL-2, IL-6, IL-12, TNF- α , IFN- γ , T-Bet, CD25, Foxp3, TGF- β , and IL-10. Results: The gene expression of T-Bet was consistently and significantly increased by exposure to rhOPN ($p < 0.01$). Densitometric analysis of gels revealed an approximate 4-fold increase in T-bet gene expression in response to rhOPN. The other molecules tested showed no significant alteration in gene expression in response to rhOPN.

Conclusions

Previous research has indicated that T-Bet is associated with T-cell activation, induction of a Th1 cellular immune response and trafficking of immune cells into inflamed tissue. Our results demonstrate an increased expression of T-Bet gene expression in response to osteopontin and suggest that rhOPN may have a pro-inflammatory effect on PBMCs. Osteopontin may thus enhance the infiltration of immune cells into the rheumatoid synovium.

No conflict of interest to disclose

A338

P123

**The Right Test for the Wrong Person at the Wrong Time -
Haemoglobinopathy Testing in the Hunter Area**

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Aim

Review indications for haemoglobinopathy testing, as performed by Hunter Area Pathology Service.

Method

A cross-sectional audit was performed of all haemoglobinopathy testing requests received by Hunter Area Pathology Service during 2007. Laboratory workflow sheets, Laboratory Information System (LIS) and area-wide patient database (Clinical Access Portal, CAP) for all haemoglobinopathy requests were reviewed.

Results

339 haemoglobinopathy requests were received in 2007. Stated indications were: microcytosis (15%, n=52); anaemia (14%, n=46); provisional diagnosis of haemoglobinopathy (36%, n=121); African refugee status (23%, n=78); no clinical information (6.5%, n=22). 71% of assays performed to confirm a provisional diagnosis of haemoglobinopathy were normal (87 out of 122). Twenty two of 31 patients (71%) who had haemoglobinopathy testing for microcytosis were found to have thalassaemia. Two patients with microcytosis were actually identified as sickle cell traits. One out of thirteen patients investigated for polycythaemia was found to have Haemoglobin Kempsey. 32% of patients investigated for microcytosis and/or anaemia had not had iron studies performed. Only one patient had haemoglobinopathy testing performed in the first trimester of pregnancy. Pre-anaesthetic assessment was not listed on any request forms.

Conclusion

Published guidelines outline indications for haemoglobinopathy testing. This audit highlights differences between such "best practice" and current practice. Census-based statistics demonstrate likely under-diagnosis of patients within the Hunter region. Important indications such as pre-natal counselling and pre-operative risk assessment are under-represented. Education of health professionals in regards to guidelines for haemoglobinopathy testing is a current and future priority aiming to minimise morbidity and mortality associated with Haemoglobinopathies.

No conflict of interest to declare

A339

P124

A Study of HFE Gene Mutations in Iron Deficient Females

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Aim

Since HFE gene mutations in hereditary haemochromatosis are associated with increased iron absorption, this study was carried out to determine whether these mutations and correspondingly iron deficiency and anaemia would be less frequently observed in iron deficient patients presenting for intravenous iron therapy.

Method

Following ethics committee approval and informed consent, EDTA blood samples were obtained from 196 cases of females (ages 16 – 70 years) with iron deficiency (ferritin at referral <30ug/L), (4 cases were excluded, patient questionnaire not completed). Tests were carried out for Hb, ferritin and HFE, the latter by standard PCR methods using sequence specific primers capable of identifying 3 HFE mutations (C282Y, H63D, S63C).

Results

Heterozygous C282Y with mean Hb 112.6g/L (range 69-137g/L), ferritin 33ug/L (range 2-35ug/L) was identified in 6.8% of the group (expected gene frequency 14.1%). 80/132 (60.6%) patients with the normal allele and 5/13 (38.5%) C282Y carriers had a normal Hb.

Heterozygous H63D with mean Hb 140g/L (range 70-144g/L) mean ferritin 16.4ug/L (range 3-53ug/L) was identified in 22.4% (expected 23.6%).

66.8% of patients with wild-type HFE who acted as controls had a mean Hb of 114.95g/L (range 66-140g/L) and a mean ferritin 16.66ug/L (range 3-76ug/L). Other data to be supplied.

Using Fisher's exact test, no statistical difference was present when the Hb and ferritin of the iron deficient control group was compared with those heterozygous for C282Y and H63D.

Conclusion

The presence of the HFE gene mutations in iron deficiency does not appear to confer a superior advantage in terms of iron stores or Hb when compared to iron deficient females with a normal HFE genotype. However while not statistically significant there was a trend towards a higher Hb in the C282Y carrier iron deficient group. It is possible that this study was not powered to detect small changes and a larger survey may be necessary.

This research was supported by Clinipath. The company had no role in analysing the data or preparing the abstract.

A340

P125

Serum Prohepcidin and Ferritin in Myelodysplasia

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Aim

Hepcidin formed from its precursor, Prohepcidin is a key regulator of iron metabolism. Dysregulation of the latter and transfusion dependent anaemias together with iron overload are features of the Myelodysplastic Syndromes (MDS), in particular Refractory Anaemia with Ring Sideroblasts (RARS). The present study was organised to understand the relationship between hepcidin and ferritin and determine the value of measuring hepcidin in MDS.

Method

EDTA and serum samples were collected from 19 patients with MDS (RA 8, RARS 8, RCMD 3), 7 patients were transfusion dependent, 65 patients with miscellaneous haematological diseases and 58 female patients with iron deficiency (total, 142). Serum ferritin was measured using a chemiluminescence immunoassay ECLIA (Roche Modular E170). Prohepcidin was measured by an ELISA assay (DRG International Inc, Mountainside, NJ).

Results

23/33 patients with a raised se prohepcidin (NR: 58-158ng/ml) had a high ferritin (>450ug/l), in contrast to 18/39 who showed a low ferritin (<30ug/l). A 3 way table comparison of low, normal and high ferritins and prohepcidin was statistically significant. Scatterplot analysis revealed a Pearson r coefficient value of 0.19 in keeping with a non-linear relationship between prohepcidin and ferritin in the studied groups.

Conclusion

This study while demonstrating an association between hepcidin and ferritin failed to show a significant correlation between the two in MDS and may reflect the heterogeneity of the latter with its varying degrees of anaemia and iron overload.

This research was supported by Novartis. The company had no role in analysing the data or preparing the abstract.

A341

P127

Innovations in the Haematology Outpatient Service at Christchurch Hospital, Christchurch, New Zealand

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Introduction

I took over the position of Charge Nurse Manager in July 2006. One of my challenges in this new position was to address the issues of the Haematology Outpatient Service and implement change to improve patient flow and to develop patient centred care.

Problems

The role of the outpatient nurse who had worked in isolation for many years and had little opportunity for professional development. The service needed reviewing to accommodate the increasing number of patients being seen in Haematology Outpatients.

Solutions:

The nurse underwent a performance review. Based on the outcome of this review a plan was implemented to address the issues that were identified. The nurse was booked in and has attended a number of professional development courses. This is an expectation of the nurse to fulfil the competencies of practice outlined by the Nursing Council of New Zealand. The nurse was also mentored by both the Clinical Nurse Specialist in the Bone Marrow Transplant Unit and the Haematology Day ward.

A business proposal was developed by me and the Clinical Nurse Specialist in the Haematology Day Ward and this is currently being implemented to initiate change. This business proposal includes addressing the lack of space, introducing Nurse led education session and improving patient flow between the Haematology Outpatient Service and the Haematology Dayward.

Conclusion

This is an exciting time for the service however, as with any change there have been challenges. There has been compromise made to ensure that this area continues to develop and meet changing needs of the service.

A342

P128

Role of Flowcytometry in the Diagnosis of Chronic Lymphoproliferative Disorders: An Indian Perspective

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Objective

The diagnosis of chronic lymphoproliferative disorders (CLPD) requires analysis of multiple parameters: cell morphology, tissue histopathology, immunological markers, molecular/cytogenetic investigations. There is paucity of information regarding diagnostic and prognostic parameters of CLPDs from Indian sub-continent.

Study Design

A prospective study was carried out to analyze the peripheral blood, bone marrow aspirate morphology, trephine biopsy infiltration pattern and lymphoid cell flowcytometry (FCM) findings in CLPD patients. These findings were compared with few other disease parameters.

Results

CLPDs comprised of 11.03 % of hematological malignancies diagnosed between January 2005 and December 2006 at our institute. Chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL) were the common CLPDs out of a total of 91 patients. According to morphology and flowcytometry, 70 cases were diagnosed as CLL, 14 as HCL and rest of the 7 cases included in 'miscellaneous' category. Sixty nine CLL cases showed typical CLL immunophenotype. All 14 but one HCL case revealed typical HCL immunophenotype. The CLL cases were divided according to Rai and Binet staging systems, and correlation of various stages with other prognostic parameters like trephine biopsy patterns, CD 38 positivity, serum lactic dehydrogenase (LDH) level, lymphocyte doubling time (LDT) and Coomb's test was analyzed. The trephine biopsy pattern showed significant statistical association with staging systems ($p=0.016$ and $p=0.005$). The other prognostic markers did not show significant statistical association with staging systems hence their role as independent markers cannot be excluded.

Conclusion

The advantages of FCM are being realized increasingly in India, therefore this communication is likely to serve as a baseline data in our setup.

No conflict of interest to disclose

P129

Chronic Falciparum Malaria

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Infection with *Plasmodium falciparum* is well known as a cause of acute malaria among travellers from endemic areas, such as Africa and South-East Asia.

Chronic infection may occur in endemic areas among those with a degree of partial immunity, and cases have been reported in people who left an endemic area up to 5 years previously. We report a case of chronic falciparum malaria in a 15 year-old boy who had not been out of Australia for 5 years, had not been near an airport nor had overseas visitors. Our patient had lived in Africa some years ago and had not returned since.

Many immigrants are asymptomatic *P. falciparum* carriers . Their immunity probably prevents clinical symptoms . In the absence of reinfections their immunity would decrease and symptoms would occur. The patient is HIV positive. In patients with HIV, susceptibility to malaria and parasitaemia increase as immune responses fail. The patient's "apparent" neutropenia is also discussed. Neutropenia in African patients should not be assessed according to Caucasian reference ranges. The neutrophil count is significantly lower in black Africans than in Caucasians and other ethnic groups.

Neutropenia due to increased neutrophil margination can be seen in malaria. The neutropenia here may be multifactorial including ethnic neutropenia, increased neutrophil margination and marrow suppression by HIV +/- HIV therapy.

In conclusion, the patient reported is a HIV positive African man who presents with chronic falciparum malaria and ethnic neutropenia.

No conflict of interest

A344

P130

Unsuspected Histopathological Lesions in Bone Allografts

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Background and Aim

Bone donors are predominantly elderly patients undergoing elective hip surgery. Most blood donor selection criteria apply. Other evaluation, including histopathological examination of bone, is also done. These were reviewed to determine occult lesions in bone in these subjects.

Methods

Retrospective analysis of bone allograft histopathology reports at Christchurch Bone Bank between 2006 and 2007. Donors had no obvious/known haematological disease. Decalcified, paraffin-embedded bone core biopsies and synovial specimens stained with H and E were examined.

Results

There were 489 specimens from 465 patients including 24 from 12 subjects who had bilateral surgery. Donor age ranged from 24 – 92 years (mean 66 y). 54.2% were females and 45.8% males. 448 (91.6%) specimens showed no abnormalities (other than osteoarthritis) while 29 (5.7%) did. None showing an abnormality was from a patient who had bilateral surgery. 13 (2.6%) specimens failed for technical reasons. Overall, the mean age of both those with and without abnormalities was 66 years. 12/29 (41.3%) abnormalities were in subjects > 70 years. The youngest donor with an abnormality was 42 years. 20 (4.0%) of specimens showed increased lymphocytes / plasma cells such that a lymphoproliferative or autoimmune disorder could not be excluded (mean age 65 y). In 5/20 there were paratrabeular lymphocyte collections. 3 (0.6%) had features suggestive of myeloproliferative / dysplastic disorders (mean age 64 y). 6 (1.2%) had other abnormalities (mean age 71 y) - 2 had crystal aggregates consistent with pseudogout or chondrocalcinosis, 2, non-necrotising granulomas and 1, an acute and chronic inflammatory infiltrate. No specimen showed avascular necrosis (though 7 had radiological evidence), tumour or metabolic bone disease.

Discussion and Conclusions

As expected and similar to the few reports on the subject, our results show that unsuspected histopathological abnormalities in bone occur in older subjects some of whom may have unrecognised, possibly pre-clinical haematological disease.

No conflict of interest

A345

P131

A Case of EDTA and Temperature Dependent Pseudo-neutropenia

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We present a case of pseudo-neutropenia due to EDTA-dependent neutrophil aggregation in a 47-year-old man with chronic renal failure. The patient first presented in 2002 with idiopathic end stage renal failure. A haematology referral was made in 2008 for investigation of long standing neutropenia. The patient's neutrophil count over this time had ranged from 0.2 to 0.8 x 10⁹/L, as assessed by an automated full blood analyser (Abbott Cell-Dyn 4000). There was no history of recurrent infections. The neutropenia had been previously investigated in 2005, including autoimmune studies and a bone marrow biopsy, with no cause identified.

In the most current investigation, the patient's peripheral blood film revealed the presence of numerous small neutrophil aggregates. Retrospective review of the available previous films showed this to be a consistent unreported finding. To further investigate the phenomenon, we performed a fresh 37°C blood collection in both EDTA and citrate collection tubes. The immediate automated neutrophil counts at 37°C were 2.34 x 10⁹/L in EDTA, and 2.52 x 10⁹/L in citrate. Allowing the samples to cool to room temperature for 1 hour, decreased the neutrophil count to 0.71 x 10⁹/L in EDTA, with no change in the citrated specimen. There was incomplete restoration of the neutrophil count on rewarming the EDTA sample to 37°C. Patient serum was further investigated using the Granulocyte Immunofluorescence Test (GIFT) and the Granulocyte Agglutination Test (GAT). Both the GIFT and GAT were positive using a granulocyte panel from four random blood donors.

In this case we have demonstrated EDTA- and temperature-dependent neutrophil aggregation, associated with the presence of anti-neutrophil antibodies in patient serum. Although pseudo-neutropenia is rare, the case reinforces the importance of careful blood film examination in preventing unnecessary and invasive investigations due to spurious automated counts.

No conflict of interest to disclose

A346

P132

Disease Characteristics and Flowcytometric Analysis of Paroxysmal Nocturnal Hemoglobinuria (PNH) cases from North India

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Introduction

Classical Paroxysmal nocturnal hemoglobinuria (PNH) is diagnosed in the setting of strong clinical suspicion, using certain conventional and newer tests. Conventional tests include Ham's acidified serum test (HAST), sucrose lysis test (SLT) and urine hemosiderin. In addition flowcytometric (FCM) analysis of RBCs and WBCs is performed using anti – CD 55 and anti – CD 59 monoclonal antibodies (moab).

Aim

To analyze the disease characteristics and flowcytometric results of classical PNH cases.

Method

Between January 98 and December 2006, tests for PNH were conducted for 198 adult patients in our institute. Clinical details, blood counts and bone marrow findings were noted, in addition to the results of conventional and FCM tests.

Result

Thirteen cases fulfilled the diagnostic criteria of classical PNH. FCM, HAST and SLT were positive in all. Presenting complaints mostly related to cytopenia/s and intravascular hemolysis; only one patient suffered thrombotic event. Median duration of onset of symptoms to diagnosis and to last follow up was 3 years (range 6 months – 10 years) and 4.5 years (range 12 months- 11.4 years) respectively. Bone marrow aspiration and trephine biopsies were hypercellular in 9, normocellular with variable cellularity in 3 and hypocellular in 1 patient. Erythroid hyperplasia was observed in all. Using FCM, three PNH cell populations were detected in 5 patients (38.46%), rest 8 showed two PNH cell populations. PNH III populations in the RBCs and neutrophils ranged between 7-60% and 13-61% respectively.

Conclusion

Different composition of PNH cell types (a mixture of type I, II and III cell populations in 38.46% patients in our study) and rarity of thrombotic events emerge as important features of PNH patients diagnosed at our institute. It is important to make a distinction between classical PNH and 'PNH defect' detected in other hematological disorders.

No conflict of interest to disclose

A347

P133

Interpretation of Full Blood Count (FBC) – Results of a Survey at the Australian National University (ANU) Medical SchoolDavid Owen¹ and Dipti Talaulikar^{1,2}¹ANU Medical School, ²Department of Haematology, The Canberra Hospital.**Introduction**

With the introduction of problem-based learning (PBL) in medical curricula, students are called upon to interpret investigations such as Full Blood Count (FBC) early on. This study aimed to assess situations in which students at ANU Medical School were required to interpret FBC, and their ability and level of confidence.

Methods

Separate student and staff e-surveys were used; student surveys were designed to assess situations in which students required to interpret FBC, determine ability using an optional problem solving exercise, and perceived level of confidence. Staff surveys aimed to assess perceptions on students' abilities in using this skill.

Results

Of 72 student respondents, more [70 (97.2%)] reported using FBC interpretation within PBL sessions than within clinical situations [35 (48.6%)]. Some degree of difficulty in interpretation was reported by 52 (76.4%). Of 66 (91.7%) students who answered the optional interpretative question, depth of interpretation varied with 65 (98.5%) identifying anaemia, 38 (57.6%) microcytic anaemia, and 17 (25.8%) microcytic hypochromic anaemia. Forty three (65.2%) provided one, and 31 (47%) two correct differentials. Using a 1-5 scale confidence scale (5 most confident), 24/68 (35.3%) respondents scored 3, 26 (38.2%) scored <3 and 18 (26.5%) >3. Academic staff surveyed (n=25) gave non-committal (9), categorical positive (4), categorical negative (4) and conditional negative responses (8) regarding student skills in FBC interpretation.

Conclusions

Students are required to interpret FBC early on within new medical curricula. A large proportion of students report difficulties with this skill. Although simple cases are solved with ease and confidence by students, greater effort is required to improve depth of interpretation, and level of confidence.

No conflict of interest to disclose

A348

P134

A Study in the Use of Recombinant Human Haemopoietic Growth Factors to Improve Myeloid Cytogenetic Cultures: A Western Australian Experience

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The proliferation, maturation and survival of bone marrow precursor cells in vivo require the presence of specific growth factors. The aim of this study was to improve the mitotic index and chromosome quality of our current bone marrow cultures using growth factors added in vitro.

To achieve this a combination of recombinant human haemopoietic growth factors (GF) were added to the culture medium. The growth factors used were granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, stem cell factor and interleukin-3. This combination was selected based on previous published studies.

Twenty four-hour short-term synchronised cultures were performed in RPMI supplemented with 20% bovine serum. Forty four paired parallel bone marrow cultures were analysed in a blinded fashion and the GF cultures compared with the current (reference) cultures.

Cultures containing the growth factors had an improved banding score and higher mitotic index, whilst maintaining the same percentage of abnormal cells when compared to the reference cultures. This improvement in quality should result in optimisation of time taken for slide making, metaphase selection and analysis of both normal and abnormal cells. This should have efficiency and cost savings for the laboratory. The improved chromosome quality should enable the detection of additional chromosome abnormalities over time.

No conflict of interest to disclose

P135

Response of E459K BCRabl Mutation to Tyrosine Kinase Inhibitors**Kelly Emmanuel**, Melinda Higgins, Paul Cannell*Haematology Department, Pathwest, Royal Perth Hospital, Perth, Western Australia***Aim**

The first generation Tyrosine Kinase Inhibitor (TKI) Imatinib is now the treatment of choice in Chronic Myeloid Leukaemia (CML). However many patient's develop resistance, most commonly through mutation of the ABL kinase domain that disrupts drug binding or favours the active conformation of the kinase. Second generation TKI's such as Dasatinib and Nilotinib have been developed to overcome resistance but have varied activity on the different mutations. Extensive in-vitro and in-vivo studies have been performed to ascertain the drug sensitivity pattern of each mutation to aid treatment, but many mutations remain uncharacterised. We present two case reports as evidence of the in-vivo resistance of mutation E459K to Imatinib and subsequent sensitivity to Dasatinib.

Method

Q-PCR using Taqman technology on the Applied Biosystem 7500 Sequence detection System was used to monitor BCR-abl levels in two CML patients at various time intervals to monitor disease response to treatment. Loss of response to treatment was investigated by performing mutation studies on the ABL kinase domain using nested PCR and sequencing with Applied Biosystem BigDye V3.1 chemistry and 3130 PRISM instrument.

Results

Two patients with varied clinical histories developed resistance to Imatinib therapy and entered molecular relapse. BCRabl mutation studies revealed E459K as the sole mechanism of resistance. Both underwent a change of treatment to Dasatinib whereby they demonstrated a rapid decrease in disease, achieving a major molecular response.

Conclusion

We present two case reports as evidence of the in-vivo sensitivity pattern of BCRabl mutation E459K, aiding clinical use of first and second generation TKI's against this mechanism of disease resistance in CML.

No conflict of interest to disclose

A350

P136

Pauci-immune Crescentic Glomerulonephritis Associated with Bortezomib for Myeloma and Amyloidosis

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A 66-year-old man was diagnosed with renal and cardiac AL amyloidosis, without evidence of myeloma, in 2004, and treated with 12 cycles of monthly melphalan followed by autologous stem cell transplantation. He relapsed in April 2007, after a plateau phase of 8 months, and was treated with thalidomide, to which he responded well. In March 2008 he was noted to have worsening paraproteinaemia, with a monoclonal IgG kappa band rising from 1 to 34 g/L over two months. This was associated with hypercalcaemia and acute renal failure. Investigations demonstrated myeloma, with a bone marrow plasmacytosis without evidence of amyloidosis. He was commenced on bortezomib (1.3 mg/m² on days 1, 4, 8 and 11, of a 21-day cycle) and dexamethasone 20 mg weekly. After two cycles, his serum calcium and renal function had normalised and his monoclonal IgG kappa band had decreased to 19 g/L. The patient was hospitalised after cycle 2 with abdominal pain and found to have microscopic glomerular haematuria. Although the serum creatinine was normal, renal biopsy demonstrated a pauci-immune crescentic glomerulonephritis. The patient's renal function progressively worsened despite cessation of the bortezomib, a pulse of intravenous methylprednisolone (500 mg daily for 3 days) and oral cyclophosphamide-prednisolone therapy. Six weeks later he became anuric and haemodialysis-dependent. Renal amyloidosis has a rare association with pauci-immune crescentic glomerulonephritis, but almost all reported cases have occurred in the context of rheumatoid arthritis. However, in Phase I trials of bortezomib, one report of proliferative glomerulonephritis with acute renal failure was described.

No conflict of interest to disclose

A351

P137

Comparison of Serum Free Light Chain Assessment to Urinary Protein Electrophoresis in the Diagnosis of Monoclonal Gammopathies – an Updated Report from Sir Charles Gairdner Hospital, Nedlands, Western AustraliaRH Banh^{1,2}, DJL Joske^{1,2}, GM Cull^{1,2}, J Finlayson¹, J Josephs¹ and BM Augustson^{1,2}

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Aim

The serum free light chain (SFLC) assay is reported to have increased sensitivity to electrophoretic techniques in detecting light chains. It is currently used to diagnose and monitor response to therapy in light chain monoclonal Gammopathies, with the potential to replace urine electrophoresis (UPEP). We undertook a comparison of serum protein electrophoresis (SPEP) and SFLC in combination, to our current institutional practice of SPEP and urine protein electrophoresis ± immunofixation (IFE) for diagnosing monoclonal gammopathies. Here we present an analysis of data collected over 6 months.

Method

Patients from our institution previously undiagnosed but having a history suggestive of a monoclonal gammopathy were included. Samples without a direct request for SFLC were identified by review of routine requests for SPEP or UPEP. Quantitation of SFLC was by nephelometry on a Dade Behring BNII Nephelometer. Serum and urine electrophoresis was performed on the SPIFE 3000™ (Helena Laboratories) with scanning densitometry using QuickScan 2000. Inpatient notes were obtained and reviewed to determine eventual diagnosis where possible.

Results

Between 1 March and 31 August, 2007, 215 cases were identified. Fourteen percent (31/215) had paraprotein by SPEP and 15% (33/215) an abnormal κ/λ ratio (κ/λ) ($N=0.26 - 1.65$). In 63 cases (29%) a urine specimen was obtained of which 22% (14/63) had an M-protein. In 12 cases, the κ/λ was abnormal without an M-protein by SPEP – none were monoclonal gammopathies, 1 had CLL, 2 NHL, 4 renal dysfunction and the remainder were non-haematologic disorders. In 6 cases, SPEP identified an M-protein without the κ/λ being abnormal – all were cases of MGUS. The SFLC alone was able to identify all 9 new cases of MM and 6/12 cases of MGUS. There were no cases in which the UPEP detected an abnormality without either the SPEP or SFLC assay being abnormal.

Conclusion

SPEP + SFLC is a promising alternative in the initial work-up for monoclonal gammopathies. Our results are consistent with those reported in the literature but are limited by small numbers and limited follow-up from diagnosis. Additionally, all tests were from one test-kit batch and an assessment of batch to batch variation cannot be made.

This research was supported by InVitro Diagnostics Pty. Ltd/The Binding Site. The company had no role in analyzing the data or preparing the abstract.

A352

P138**Decreased Frequency or Cessation of Zolendronate Does Not Impact on C-Telopeptide Levels in Multiple Myeloma Patients****Briony A Cutts¹, Hans Schneider², Andrew Spencer¹**

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Aim

Intravenous Zolendronate (a bisphosphonate therapy) significantly reduces osteolytic progression and improves quality of life in multiple myeloma patients. It is usually administered monthly based on trial data. There is no set duration of treatment. C-Telopeptide is a useful marker of bone resorption. Our aim was to determine if C-Telopeptide levels were altered in patients whose zolendronate treatment was changed from monthly to three monthly administration or ceased altogether.

Method

We conducted a retrospective analysis over a period of 24 months on a group of nine patients. We measured their C-Telopeptide levels after patients had zolendronate ceased or changed to 3-monthly infusions. Five patients had zolendronate ceased and four patients had zolendronate changed to 3-monthly administration over this time period. C-Telopeptide levels were correlated with paraprotein measurements and the presence of lytic lesions was noted.

Results

The median age of the patients was 61. Four patients were male and five were female. Eight out of nine of the patients had lytic disease at diagnosis. Seven patients had IgG Kappa; one had IgG Lambda and one patient had Kappa free light chain multiple myeloma. The median C-Telopeptide level in patients changed to 3-monthly zolendronate was 68.4pg/ml. This was similar to patients who had zolendronate ceased where the median C-Telopeptide level was 105.5pg/ml. (Normal C-telopeptide levels are < 700 for men and women aged 60). No patient had worsening paraprotein levels or new lytic lesions.

Conclusion

Our results suggest that three monthly administration of zolendronate or cessation in stable patients does not impact upon bone resorption based on measurements of C-Telopeptide. A larger randomised trial measuring C-Telopeptide levels and comparing monthly with three monthly infusions would be useful to further prove these observations.

No conflict of interest to disclose

A353

P139

Lymphoid Subsets and Regulatory T Cell Profiles in Patients with Relapsed Multiple Myeloma in a Subset of Patients Enrolled in the REVLITE Trial

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Aim

The profile and impact of altered lymphocyte subset (LSS) and regulatory T-cells (Treg), in multiple myeloma (MM) is controversial. Moreover, how they change with immunomodulatory drug (IMiDs) treatment is unclear. We aim to correlate LSS and strictly defined Treg (CD4⁺CD127^{dim}FOXP3⁺CD25⁺) profiles of patients with relapsed/refractory MM with disease response during lenalidomide (len) treatment.

Method

Sixteen out of 40 patients have been recruited into the phase II single-arm trial (REVLITE) of low-dose len (15mg d1-21q28) and dexamethasone (20mg/d, d1-4,9-12, 7-10). Baseline LSS and Tregs were measured by flow cytometry, and compared to 10 age-matched healthy donors. Subsequent serial assessment with each treatment cycle was done. The international uniform response criteria were used.

Results

After a median of 4 cycles (range 2-9), 13 patients had a response (1 IF-negative CR, 12 PR). At baseline, total CD3⁺ T-cell (6.1 vs. 11.2 x10⁵/ml; p=0.007), CD4⁺ T-cell (2.0 vs. 9.7 x10⁵/ml; p<0.00005), Treg (0.39 vs. 4.3 x10⁴/ml; p=0.0003), and B-cell (0.76 vs. 3.2 x10⁵/ml; p=0.05) numbers were lower in patients compared to healthy controls. Conversely, CD8⁺ T-cell numbers were conserved (3.2 vs. 2.7 x 10⁵/ml; p=0.28) and activated cytotoxic T-cells (CD8⁺CD57⁺) were higher in MM patients (2.1 vs. 0.68x10⁵/ml; p=0.01). In 10 patients completing at least 4 treatment cycles, no recovery in total T or B-cell numbers were observed despite all achieving at least a PR. Treg numbers increased after 4 cycles (0.12x10⁴/ml at baseline vs. 1.16x10⁴/ml, p=0.005), but still remained below normal range. Correlation with final clinical outcome is yet to be assessed.

Conclusion

Patients with relapsed/refractory MM have low CD4:CD8 ratios and depressed Treg and B-cell numbers which, despite clinical responses, have not normalised after 4 len-dex cycles. Treg recovery in responding patients is seen, albeit incomplete, suggesting that Tregs play an important role in the modulation of tumour micro-environment. Longer-term follow-up in larger patient numbers will be revealing.

The REVLITE trial is an investigator initiated study but was partially supported by funding from Celgene. The company had NO role in analysing the data or preparing the abstract.

A354

P140**Trogocytosis in Multiple Myeloma**

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Aim

Trogocytosis is the transfer of cell membrane material from one cell to another during cell-cell contact. Recent studies have suggested that the transfer of material across the immunological synapse can alter the function of T cells and even produce adaptive T regulatory cells Tregs. We sought to identify the extent of trogocytosis in patients with multiple myeloma and to determine whether this process is at least partly responsible for immune tolerance.

Methods

An in vitro model of trogocytosis was established in which plasma cells were biotinylated and then cultured with patient mononuclear cells. Flow cytometry was used to determine cell surface expression of membrane proteins.

Results

Plasma cells (CD38++) were easily biotinylated and trogocytosis occurred with 36% of T cells but not B cells in the in vitro culture model. Of the antigen presenting cell molecules at the immunological synapse (CD80, CD86, B7-H1, B7-H3 and PD-L2) only CD80 and CD86 showed significant transfer to T cells. HLA-G expression on T cells was less than 1% on 69/70 different samples. CD80 expression was found on the T cells of 9% of patients and CD86 was found on 13% of patients (n= 95). Both CD4 and CD8 memory (CD45RO+) cells were involved but not naïve T cells. In vitro stimulation and mRNA studies showed that T cells acquire cell surface antigen but do not produce CD80 and CD86 mRNA. No trogocytosis was evident on age-matched controls.

Conclusions

T cell trogocytosis is common in patients with multiple myeloma. Transfer of proteins from tumour cells to T cells may alter T cell function and provide a mechanism of immune tolerance for the tumour.

No conflict of interest to disclose

A355

P141**Outcome of High-Dose Chemotherapy and Autologous Peripheral Blood Stem Cell Transplantation for Multiple Myeloma at Waikato Hospital**

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High-dose chemotherapy and autologous peripheral blood stem cell transplantation (SCT) is considered the standard of care for newly diagnosed patients with multiple myeloma (MM) up to 70yo. A retrospective audit was undertaken to assess the efficacy and outcome of a cohort treated on a uniform Mel200 protocol at a single institution over 11 years.

All patients who received their first SCT for MM at Waikato Hospital between 1996 and June 2007 were included; second SCTs were not. 56 pts were identified, 30 females / 26 males, median age at SCT 55.8 (range 32.5-68.1). Paraprotein subtypes were 28 (50%) IgG, 10 (18%) IgA, 14 (25%) light chain and 4 (7%) IgD or multiple plasmacytomata. Induction chemotherapy was CVAMP in 43 (77%), with CVAD, Cy-Dex and VBMCP in the remainder. Stem cell mobilisation was cyclo-GCSF; conditioning Mel200 +/- methyl-prednisolone.

All patients engrafted; neutrophils >0.5 median 11d (9-18), platelets >20 median 13d (8-29). Median length of stay was 19d (13-39). All-cause mortality at 100d was 1pt (2%). Assessment of response post-SCT was incomplete in many cases; however 98% (49/50 informative pts) achieved PR or better.

Median follow-up was 36 months and 13pts have died; overall survival (OS) was median 109mo (9yrs). Event free survival (EFS, relapse or death) was median 58mo. Subsequent second malignancy was seen in 2pts.

These data confirm excellent outcomes from SCT for MM at Waikato Hospital, with low mortality, brisk engraftment and highly satisfactory EFS and OS.

No conflict of interest to disclose

A356

P142**Autologous Stem Cell Transplant in AL Amyloidosis: Data on 4 Cases from a Haematology Unit in Western Sydney****Akash Kalro**, John Taper*Haematology Department, Nepean Hospital, Kingswood, Sydney***Introduction**

AL amyloidosis is characterized by tissue deposition of light or heavy chain with associated organ dysfunction. Recent data on the use of autologous stem cell transplant has shown limited benefits with respect to disease outcome¹. We present data on 4 cases treated since January 2007.

Methods

At diagnosis, all patients had a blood count, biochemistry, bone marrow, fat pad biopsy (stained with Congo Red) and 2Dechocardiography for interventricular septal thickening. Stem cell harvest was done after cyclophosphamide and GCSF mobilization. Melphalan 200mg/ m² conditioning was used. Pegylated GCSF for neutrophil recovery. Disease monitoring was with free light chain assay.

Results

The age range was between 43 – 61 years. 3 are male and one is a female. 2 patients had myeloma with amyloidosis and 2 had primary AL amyloid. All patients had cardiac and renal involvement. The range of interventricular septal thickness was between 14 - 17 mm. Post stem cell infusion the median period of engraftment was 14.5 days (10 – 17 days).

Peri transplant period in one patient was complicated by spontaneous splenic rupture² and CMV reactivation. All patients had febrile neutropenia as a complication. All patients are surviving at present, median of 6 months (range 1.5 – 18 months). Immunological remission was documented with a normal free light chain ratio in all of them.

Conclusion

Optimal therapy of AL amyloidosis is not clear. Our 4 patients with cardiac amyloid and preserved performance status, tolerated the procedure and had rapid reduction in amyloid light chain. In our opinion autologous stem cell transplant still remains a therapeutic option for patients with amyloid.

No conflict of interest to disclose

A357

P143

Assessment of Outcome of Tandem Autologous Stem Cell Transplantation for Patients with Multiple Myeloma

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Autologous Peripheral Blood Stem Cell Transplantation (APBSCT) after a conditioning regimen with Melphalan is considered the standard consolidation therapy for Multiple Myeloma (MM). Tandem APBSCT has been increasingly used to treat MM with the goal of prolonging disease-free survival (DFS), especially in patients who achieved only a partial remission.

We prospectively analysed the outcome and prognostic factors of 17 patients with MM treated with tandem ABSCT during the period from March 2006 to July 2008 at the Launceston General Hospital, Australia. The male-to-female ratio was 15:2, and the median age was 57 years (range:37-70). There were 12 patients with IgG myeloma, 1 with IgA myeloma and 4 with light chain disease. Median time from diagnosis to the first transplant was 6 months (range: 4-36). Two of these patients had relapsed disease prior to the tandem therapy.

All patients were analysed for unfavorable cytogenetic risk using PCR and FISH at our laboratory in UTAS. Sixteen of 17 patients underwent a tandem transplant while 1 had only one transplant, due to a generalised herpes zoster infection after the first transplant. Most of patients (11) received VAD induction chemotherapy, 1 patient received Velcade containing regimen and 5 patients received Thalidomide therapy. All patients underwent stem cell mobilisation with Cyclophosphamide and G-CSF.

After a median follow up period of 16 months (range:4-28), all patients were alive and 16 remained in remission (2 CR, 14 PR), while one of the relapsed MM patients showed further disease progression and is currently being treated successfully with Lenalidomide.

In summary, our analysis shows that a significant number of patients with MM derived DFS benefit from upfront treatment with tandem autologous PBSCT, which was well tolerated. Further randomised studies to compare the effect of tandem or single APBSCT with the novel chemotherapeutic agents for MM are warranted.

The authors confirm that there is no conflict of interest in relation to this research.

A358

P144

Spontaneous Rupture of Spleen Following Autologous Stem Cell Transplant for Myeloma with Amyloidosis: A Case Report

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Introduction

Myeloma can be associated with amyloidosis. Amyloidosis can complicate stem cell transplant recovery. We present a unique complication, which involved a spontaneous splenic rupture coinciding with neutrophil recovery after a stem cell transplant.

Case

A 62-year-old man with a history of myeloma diagnosed in August 2007, which was treated with Thalidomide and dexamethasone for 5 months, which gave a good partial response, was autografted for his myeloma in Feb 2008. Blood film since diagnosis always had features consistent with hyposplenic state, with an intact spleen documented on imaging which was consistent with amyloid. He had evidence of amyloid as documented on a bone marrow biopsy, abdominal fat pad biopsy stained for Congo red. He was harvested after cyclophosphamide and GCSF mobilization and the procedure was uneventful. A cell dose of 12.6×10^6 /kg of CD34 cells were obtained. He was autografted with melphalan 200-mg/ m² and he received pegylated GCSF for his neutrophil recovery on day +1 post stem cell infusion. His ensuing neutropenia was complicated by febrile episode and was managed by broad-spectrum antibiotics.

On the day+10 there was evidence of neutrophil recovery with ANC of 1.0×10^9 /L. On day+14 he developed acute abdomen with hypovolemic shock. His FBC at that time had revealed Hb of 70g/L, decreased by 20 g/l from previous days results. CT scan at that time revealed hemoperitoneum with a ruptured spleen. An urgent laparotomy and splenectomy was performed. He was managed in ICU with blood product support. He recovered uneventfully from his surgery. Histologically amyloidosis was confirmed in the spleen. He subsequently had mucositis possibly attributed to CMV reactivation, which was managed by ganciclovir.

The patient was subsequently discharged on day +37. He continues to be well; he has had a complete response from his autograft as documented by normal FLC ratio and a bone marrow plasma cell percentage of 2%.

Conclusion:

Spontaneous splenic rupture is a well documented complication described in literature as a complication of amyloid infiltration₁. It can happen during GCSF mobilization or during neutrophil recovery and should be monitored for in a transplant setting.

No conflict of interest to declare

A359

P145

Severe Factor V Deficiency Associated with AL Amyloidosis: Good Response to Therapy With Cyclophosphamide, Thalidomide and Dexamethasone (CTD)

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Clinical Summary

A 39 yr old man underwent elective cholecystectomy at which gross hepatomegaly, abnormal LFTs and perioperative bruising was noted. Liver and bone marrow biopsies confirmed amyloid deposits. Serum and urine paraprotein assays were negative, but serum κ free light chains (FLC) were marginally elevated (78 mg/L) and the marrow biopsy showed elevated clonal plasma cells (9%). INR was prolonged (3.2) and individual factor assays confirmed severe isolated Factor V deficiency (0.03U/ml; Factor X 1.27 U/ml) and no evidence of a circulating Factor V inhibitor. Further organ assessment indicated predominant extensive hepatic and renal involvement, but cardiac echo and biomarkers were normal (ALP 2515 U/L; albumin 14 g/l; urinary protein 13.6g/24h; creatinine 138 μ mol/L). There was no family history of similar presentations and genetic testing for familial amyloidosis was negative; these findings, along with the phenotype, confirmed a diagnosis of systemic AL amyloidosis. He was treated with 1 cycle of VAD (Vincristine, Adriamycin and Dexamethasone) and then 5 cycles of CTD which he tolerated well. Rapid, marked improvement in organ function has resulted, as illustrated by current selected laboratory parameter values (INR 1.2; Factor V 0.58 U/ml; ALP 255 U/L; albumin 33 g/l; urinary protein 3.93g/24h; creatinine 104 μ mol/L; serum κ FLC 15 mg/L; BM plasma cells 2%).

Conclusion

Isolated coagulation factor deficiencies have been described infrequently in patients with AL Amyloidosis and, when present, are usually of factor X. We describe a rare case of systemic AL amyloidosis associated with severe Factor V deficiency. The patient has shown an excellent early response to therapy with CTD, a recently described and well tolerated regimen. The marked improvement in organ function makes further therapy, for example with high dose melphalan, a safer proposition.

No conflict of interest to disclose

A360

P146

Light Chain Deposition Disease – A Rare Presentation with Predominant Hepatic Involvement

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Background

Light chain deposition disease (LCDD) is an uncommon clonal plasma cell disorder, with predominant kidney involvement. We present a case of LCDD associated with chronic lymphocytic leukaemia, manifesting as liver involvement with unexplained bleeding tendency.

Case Report

A 68 year old man was referred in June 2007 with transient lymphocytosis ($4.3 \times 10^9/L$) of classic CLL immunophenotype. Examination revealed no lymphadenopathy or splenomegaly but gross hepatomegaly which was homogeneously enlarged on CT. Serum showed 2g/L IgG kappa, immunoparesis and mildly abnormal liver function (GGT 164U/L, ALP137U/L). He received no treatment and failed further follow up. Ten months later he experienced persistent bleeding post TURP requiring 8 units of packed cells with no surgical cause found. Liver function showed biochemical deterioration but eGFR, coagulation profile and platelet aggregation were normal. Collagen binding to von Willebrand antigen ratio was persistently reduced. Bone marrow biopsy demonstrated extensive non-congophilic deposits, nodular lymphoid aggregates and a plasmacytosis. The deposits were histochemically consistent with light chains. Serum paraprotein was unchanged, free light chain assay demonstrated a $\kappa:\lambda$ ratio of 13.4 (RR 0.3 - 1.7) and he was nephrotic (1121mg/L) with 5% intact IgG κ and 10% free kappa light chains. Skeletal survey, sestamibi and MRI scans showed no bony disease.

Discussion

LCDD is distinguished from amyloid on biochemical, histological and clinical characteristics. Presentation is most commonly nephrotic, but heart and liver may be affected. In comparison to amyloid, only one report of concurrent bleeding diathesis was identified in the literature. Given two prior reports of poor outcome with treatment of hepatic LCDD with infusional VAD (rapid liver failure and liver rupture) the patient has been commenced on lenalidomide.

No conflict of interest to disclose

A361

P147

Successful Therapy for Intravascular Large B Cell Lymphoma with Immunochemotherapy**Ashish Bajel**¹, Anthony Longano², Huyen Tran¹, Stephen Opat¹¹*Department of Haematology, Monash Medical Centre, Clayton, Victoria, Australia.* ²*Department of Anatomical Pathology, Monash Medical Centre, Clayton, Victoria, Australia***Aim**

Intravascular Large B cell lymphoma (IVLBCL) is a rare extra nodal lymphoma characterised by selective growth of lymphoma cells in the lumina of small vessels. Given the rarity of this entity, the optimal therapeutic strategy is unknown.

Method

We report two cases of IVLBCL successfully treated with rituximab based chemotherapy and review the literature for further information regarding treatment.

Results

Case I – A 59 year old caucasian male presented with generalized seizures and worsening mentation. He was initially diagnosed with acute demyelinating encephalomyelitis and treated with methylprednisolone, however failed to improve. CT scanning performed subsequently revealed bilateral enlarged kidneys with biopsy showing features of IVLBCL. Despite a poor performance status, he was treated with high dose methotrexate and R-CHOP and has had a major response (CRu) with minor residual abnormalities on MR scan. There has been no evidence of disease progression at 18 months.

Case II- A 51 year old asian female presented with pyrexia of unknown origin. She had no lymphadenopathy or organomegaly however CT scan of the chest revealed pulmonary infiltrates which upon biopsy revealed IVLBCL. She received 6 cycles of R-CHOP chemotherapy and remains in remission 10 months post treatment.

These cases illustrate the two variants of IVLBCL; the western variant commonly presents with CNS and skin involvement; the asian variant presents with fever, bone marrow involvement, hepatosplenomegaly and haemophagocytosis. Most literature on IVLBCL is in the form of case reports and small series. Earlier series have suggested anthracycline based chemotherapy to be standard treatment (3- year OS 30%) while experience with ASCT remains limited. Recently the IVL Study group (Japan) reported a significantly higher 2 year PFS (56% vs. 27%) and OS (66% vs 46%) in patients who received R-chemotherapy compared with chemotherapy alone.

Conclusion

These data suggest R-chemotherapy should be considered standard of care.

No conflict of interest to disclose

A362

P148

Follicular Lymphoma of the Gastrointestinal Tract – A Case Series of Seven Patients

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Aim and Method

Follicular lymphoma is a common indolent non-Hodgkin's lymphoma. However, primary follicular lymphoma (FL) arising from the gastrointestinal tract (GIT) is rare. We reviewed the characteristics of seven patients with GIT follicular lymphoma encountered at our institution from May 2005 to July 2008. The patients' presenting symptoms, investigations, treatment and progress are presented. A review of other studies is performed.

Results

PRESENTING SYMPTOMS: included dyspepsia (n=4), nausea (n=1), abdominal pain (n=1) and bowel obstruction (n=1). Sites of involvement included duodenum (n=6) and ileum (n=1). On endoscopy, the appearances were of a patchy or confluent nodular infiltrate of the mucosa. Biopsy revealed the typical histological and immunohistochemical features of follicular lymphoma, primarily grade 1.

STAGING: included CT scan, PET scan and bone marrow biopsy. The stage of disease at presentation was: Stage IE (n=4), stage II (n=1; abdominal lymphadenopathy) and stage IV (n=2; BM, BM+abdominal lymphadenopathy). On PET scan, only two patients had FDG uptake at the site of GIT involvement.

INITIAL TREATMENT: differed depending upon the stage (and therefore aim of treatment) and symptoms. These included: observation (n=2), R-CHOP+rituximab maintenance (n=1), R-chlorambucil/prednisolone (n=1), R-CHOP+radiotherapy (n=1), R-chlorambucil/prednisolone+radiotherapy (n=1) and to be decided (n=1).

OUTCOME: Three patients treated with chemotherapy +/- radiotherapy achieved remission. The two patients who were observed have progressed. One received radiotherapy alone and entered remission while the other elected for ongoing observation. The remaining two patients are either receiving treatment or awaiting a treatment decision. All patients are alive with a follow-up of 1-38 months with the following status: remission (n=3), slowly progressive disease under surveillance (n=2), receiving treatment (n=1) and awaiting treatment decision (n=1)

Conclusion

FL of GIT is rare. The duodenum was the most commonly involved site in our series. Presenting symptoms and endoscopic appearances are non-specific and biopsy reveals the unexpected diagnosis. Chemotherapy and radiotherapy are effective treatments. Optimal management is unknown but the unique location with attendant risk of perforation or obstruction often requires consideration of early treatment.

No conflict of interest to disclose

A363

P149

**Durable Spontaneous Remissions in Lymphoproliferative Disorders:
Two Case Reports**

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There are few reported cases of lymphoproliferative diseases spontaneously remitting. Our first case is a 59 year old woman with stage IIIA marginal zone lymphoma (MZL), diagnosed in 1980 (initially diagnosed “malignant lymphoma; nodular pattern”; this was later revised). A staging lymphogram demonstrated widespread enlargement of inguinal and abdominal nodes; LDH not tested. She was monitored and during follow-up, her lesions resolved clinically. A lymphogram showed reduction in disease bulk. Repeat nodal biopsy showed reactive changes without any evidence of malignancy. Despite follow-up, she presented in 1997 with a supraclavicular node. CT revealed pulmonary nodes and bulky pelvic disease, which was biopsied and classified as nodular lymphocyte predominant Hodgkin’s disease (HD). Original slides re-classified as HD. Six cycles of ABVD achieved CR, but she relapsed in 2005 with stage IIIA disease in a similar distribution. Repeat histology in 2005 showed MZL (1980 slides were reviewed and re-classified as MZL). The patient received six cycles of CVP-R and remains in CR.

Our second case is a 75 year old woman, diagnosed in 1988 with stage IV follicular lymphoma (FL); normal LDH and B2M. Bone marrow was involved, with a large cell component in both marrow and blood (confirmed monoclonality on flow cytometry of blood). No lymphadenopathy detected clinically. Marrow biopsy in 1992 showed spontaneous remission. In 2005 she presented with abdominal bloating and stage IIIA disease, with moderate intra-abdominal lymphadenopathy. Nodal biopsy confirmed grade 3a FL. Rituximab was commenced this year for progressive disease causing hydronephrosis, with significant reduction in lymphadenopathy. The patient remains on maintenance rituximab.

Spontaneous remissions can occur with a range of non-Hodgkin lymphomas and can be durable, as demonstrated above. The mechanism underlying this is poorly understood. These phenomena may be due to immune modulation, possibly secondary to infection. Understanding the processes underlying spontaneous remissions may help to better elucidate lymphoma biology.

No conflict of interest reported

A364

P150

Treatment Outcomes in Elderly Patients (75 years old and older) With Diffuse Large B-Cell Lymphoma (DLBCL)

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Aim

Aggressive non-Hodgkin lymphoma is a common presentation in elderly patients. Treatment decisions are difficult, as most large randomised studies have excluded patients aged more than 80 years. This study aimed to identify prognostic factors for survival and to develop treatment recommendations for the elderly.

Method

We undertook a retrospective (14 years) clinical audit of 48 elderly patients treated for DLBCL at our institution.

Result

Overall survival (OS) was correlated with age adjusted International Prognostic Index (aIPI). Four other features had a significant correlation with survival: number of days spent as an in-patient, complete remission rate (CR), delay of dose administration during treatment, and administration of Granulocyte Colony Stimulating Factor (G-CSF). Survival was similar in patients treated with intensive chemotherapy (R/CHOP) and those who received reduced intensity chemotherapy. Addition of rituximab to chemotherapy did not alter outcomes in this cohort. Consolidation radiotherapy in bulky disease improved CR. Localized radiotherapy alone provided excellent response rates in localised lymphoma.

Conclusion

We confirm that good CR rates can be achieved with both standard intensity (CHOP) and reduced-intensity chemotherapy, in the very elderly with DLBCL. G-CSF support during chemotherapy improved rates of CR and overall survival. The role of rituximab requires further study in this group of patients.

No conflict of interest to disclose

A365

P151

2 Cases of Hepatosplenic T-Cell Lymphoma in the Setting of Underlying Autoimmune Disease and Immunosuppression

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Background

Two cases of Hepatosplenic T cell lymphoma in immunosuppressed patients are reported.

Case 1

A 30yo man presented with bruising, lethargy, fever, weight loss and splenomegaly. He was found to be cytopenic, (Platelet $49 \times 10^9/L$, Neutrophils $1.7 \times 10^9/L$, Haemoglobin 118gm/L), with normal cell counts 2 months prior. There was a history of immunosuppressive use for severe psoriasis, initially with weekly methotrexate (12months), followed by Cyclosporin (4 years), punctuated by 3 months of etanercept use 2 years prior to presentation.

Bone marrow exam revealed infiltration of marrow sinuses by intermediate-sized lymphocytes in an intra-sinusoidal distribution. Immunophenotype by immunoperoxidase staining and flow cytometry was consistent with T cell lymphoma (CD2/3/7/16/56/TIA-1 POS, CD4/8/38/57/HLA-DR NEG); cytogenetic analysis found a malignant clone with add(1p), loss of (14) & (20). A diagnosis of hepatosplenic T-cell lymphoma was made on the distinct histological appearance and the immunophenotype.

Case2

A 39yo man presented with a flare in Crohn's disease, hepatomegaly and incidental neutropenia, later developing splenomegaly. (Platelet $281 \times 10^9/L$, Neutrophils $0.5 \times 10^9/L$, Haemoglobin 140gm/L) Crohn's disease was initially diagnosed 13 years prior, necessitating a bowel resection and intermittent mesalazine. A combination of azathioprine and prednisone was used for 4 years prior to presentation. Liver biopsy demonstrated an infiltrate of intermediate to large T cells in liver sinusoids. Immunophenotyping of marrow and liver tissue showed a T cell monoclonal population (CD2/3/7/16/TCR $\alpha\beta$ POS, V β 12 POS, CD4/8/38/57/HLA-DR NEG). Karyotyping revealed isochromosomes (7q), (8q) and (Y) loss. A diagnosis of hepatosplenic T-cell lymphoma was made on the distinct histological appearance and the karyotype.

Discussion

Hepatosplenic T-cell Lymphoma is a rare entity first described in $\gamma\delta$ T cells, with the $\alpha\beta$ variant later recognised. Patients tend to be adolescent and young males. Auto-immune disease and treatment with immunosuppression appears to be risk factors, with a number of series describing patients post solid organ transplants and anti-biologicals for autoimmune disease. The disease is aggressive with poor median survival of 1-2 years. There had been anecdotal reports of long term survival with intensive chemotherapy followed by autologous or allogeneic stem cell transplants.

No conflict of interest to disclose

A366

P152

A Population Based Study of the Effect of Rituximab Immunotherapy on the Survival of Patients with Diffuse Large B-cell Lymphoma (DLBCL) in Queensland

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Aim

Rituximab has improved the survival of patients with DLBCL in randomized studies. We performed a population-based analysis to see if this effect was generalisable to adults with DLBCL in Queensland.

Methods

We compared survival outcomes during a six year period from Jan 2000 to Dec 2005. Data was abstracted from a single consolidated statewide cancer database called the Queensland Oncology Repository. We defined the “pre-rituximab” and “post-rituximab” eras according to the dates of availability of rituximab on the Australian Pharmaceutical Benefits Scheme (PBS) schedule (1 July 2003 for patients ≥ 60 yrs and 1 April 2005 for patients < 60 yrs). However, since younger patients may have received rituximab before April 2005, we conducted a sensitivity analysis using a single cutoff date of 1 July 2003 to demarcate between pre- and post-rituximab eras. The effect of “rituximab era” on overall survival (OS) was assessed by the log-rank test, and through multivariate proportional hazards regression controlling for demographic variables and comorbidities.

Results

Of 1210 patients with DLBCL there were 641 patients in the pre-rituximab group and 569 in the post-rituximab group. The 2yr OS was 60% and 69% in pre-rituximab and post-rituximab eras, respectively. The difference in survival between eras remained significant ($p < 0.001$) even after controlling for comorbidities and other demographic variables such as age and sex; adjusted hazard ratio (HR) for the post-rituximab era was 0.69 (95%CI: 0.57-0.82). The effect of rituximab era on survival was still significant (HR 0.74, 95%CI: 0.62-0.89) even when the pre- and post-rituximab eras were defined using a single demarcation date of 1 July 2003. The rate of stem cell transplantation also declined at the same (11% vs 4%, pre- vs post-rituximab respectively, $p < 0.001$). Age and comorbidities also significantly impacted survival, but not residence (urban vs rural) or socioeconomic status.

Conclusion

In this population based study, the introduction of rituximab has resulted in a 9% absolute improvement in 2yr OS among Queensland patients with DLBCL.

Authors declare that there are no conflict of interests

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