Journal of the Sri Lanka College of Haematologists

Volume 5(1), December 2013

Journal of the Sri Lanka College of Haematologists

Volume 5(1), December 2013 ISSN 1391 - 7919

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Printed by
Ananda Press
82/5, Sir Ratnajothi Saravanamuttu Mawatha,
Colombo 13.
Phone: 0112435975
E-mail: anpress@sltnet.lk

Journal of the Sri Lanka College of Haematologists

Volume 5(1), December 2013

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Editorial

Integrating genetic testing to haematology practice in Sri Lanka

H W W Goonasekera

We are in the era of genomic based medicine[1]. The discovery of the Philadelphia chromosome exemplifies the importance of investigating for a genetic defect in disease[2,3]. Over the past few decades discovery of genetic basis of disease has occurred at a very rapid pace and disease specific genetic markers have been quickly incorporated into diagnostic criteria and management protocols[4]. In the field of haematology, genetic defects have made a major impact on haemato-oncology where genetic markers of clinical significance have superseded morphology as a diagnostic tool[5,6] and genetics has also become important in non-malignant haematology specialties for either diagnosis[7-9], management[10], or carrier screening and counselling[11,12].

At present Sri Lanka has the expertise to perform molecular and cytogenetic testing for haematological disorders which are offered as routine genetic diagnostic services by a government sector laboratory[13] and several private sector laboratories[14-16]. Overall they offer genetic testing for both malignant and non-malignant haematological disorders and genetic testing for thalassaemia and thrombophilia are commonly performed.

Based on research data on thalassaemia in Sri Lanka, appropriate genetic screening tests for the disorder can be selected[17,18]. The genetic thrombophilia testing is offered by genetic diagnostic services for the described common thrombophilia mutations; i.e. factor V *(F5)* – 1691G>A (rs6025), prothrombin *(F2)* - 20210G>A (rs1799963) and Methylenetetrahydrofolate reductase *(MTHFR)* – 677C>T (rs1801133). The prevalence rates of these three thrombophilia mutations in the Sri Lankan population is similar to that of other South Asian populations[19] however they differ to that of Caucasian populations[20]. Controversies exist regarding the clinical utility of genetic testing for these mutations in patients with thrombophilia[21,22].

Prior to embarking on genetic testing either for diagnosis, determination of carrier states, or medical research, the haematologists should always have to take into account the ethical issues pertaining to genetic testing such as informed consent, voluntary participation, pre and post test counselling, and confidentiality, as well as management of the data which may have psychological, social, or management implications[23, 24, 25]. It is essential to have information sheets and consent forms made to suite the population locally / at national level[26]. Genetic screening programmes need to confirm to the universal norms of population screening and give due consideration regarding selection of proper study populations, adherence to ethical principles, selection of cost effective genetic tests which are properly standardized and further more it should bring about proven benefit for the patient or study population[27]. International standards as defined by the International Organization for Standardization (ISO/IEC 17043), Clinical and Laboratory Standards Institute (MM14-A2), etc, have defined the required criteria for proficiency testing / external quality assurance in genetic testing[28,29] and this is a vital area which has to be incorporated into genetic testing laboratories in Sri Lanka. The process of obtaining ISO 15189 accreditation[30] is underway in many of the private sector and selected government sector non-genetic medical laboratories.

Foreseen future challenges to the Sri Lankan haematologists are; the formulation of haematology investigation guidelines incorporating genetic tests, especially for common conditions such as

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thalassaemia, emulating other countries which have successful screening programmes which include genetic testing[31,32] and thrombophilia which has to be made in a rational and cost effective manner. Secondly the process of development of national standards for genetic testing in Sri Lanka which can best be brought about by close liaison with the geneticists working in genetic laboratories and also the National Accreditation Authority of Sri Lanka which has a vital role to play in assisting the process of laboratory standardization and accreditation.

The haematologists play a pivotal role in laboratory diagnostics and their active participation can bring about improvement of standards and initiate cost effective utilization of genetic diagnostic services in the country. Furthermore they should take the lead in guiding policy makers to bring about greater integration between genetic diagnostics and haematology practice in Sri Lanka.

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Leading article

Integrated haematological malignancy diagnostic services; the rationale and key elements of organization

R Ireland[1]

Key words: haematological malignancy, integrated diagnostic service.

Abstract

In the United Kingdom (UK), the optimal processes of care for haematological malignancies have been defined from evidence gathered of models and quality of service in 'Improving Outcomes Guidance for Haematological Oncology 2003'[1]. The guidance included recommendations about laboratory services to improve the accuracy of diagnosis of haematological malignancies. Haematological malignancies are a complex group of neoplastic diseases as defined and characterized by the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues 2008[2]. This unified concept of haematological malignancies based on an understanding of the cellular pathology of the bone marrow and immune systems. Accuracy and certainty of diagnosis remain problematic, particularly for lymphomas, with evidence that accuracy of diagnosis is slowly improving but still only approaching 85%. There are potentially significant human and financial costs of diagnostic errors. Historical pathology practice has often resulted in the separation of laboratory haematology from histopathology and of liquid and tissue specimens. The historical, fragmented diagnostic model has been challenged and proposals made for re-integration and centralization of specialist-led, diagnostic services and reporting. This article examines the rationale, key components of organization and personal experiences of developing and implementing an integrated diagnostic service.

Introduction

Haemato-oncology services in the United Kingdom have developed through a series of service improvements initiated in 1995[3]. Subsequently the National Institute of Clinical Excellence (NICE) defined processes of care and development in 'Improving Outcomes Guidance for Haematological Oncology 2003'[1], which were designed around the patient, local multi-disciplinary teams and regional cancer networks. This was driven by the recognition that UK cancer outcomes lagged behind those achieved in comparable countries[4-6].

Current concepts of haematological malignancies are based on a unified understanding of cellular pathology of the bone marrow and immune systems. Historical pathology practice in the UK and many other countries, resulted in the separation of laboratory haematology from histopathology based on differences in handling liquid and solid tissue specimens. These historical practices led to fragmentation of laboratory processes which has recently been challenged with proposals for re-integration and centralization of specialist-led diagnostic services and reporting[7-8].

Although there is evidence that the diagnosis of lymphomas is slowly improving, diagnostic accuracy remains an on-going problem and concurrence between pathologists still only approaches 85% in two recent reviews (Personal communication Byers and Norton 2008)[9].

The original concepts, rationale, components, processes and benefits of an integrated diagnostic service are applicable to any national system and are examined in this article.

Rationale and Evidence for Integrated Diagnostic services

1. Disease complexity: Haematological malignancies are complex neoplastic diseases of the

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haemopoietic and immune systems The World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues 2008[2] identifies 12 major disease groups with over 140 sub-diagnoses. Scientific advances have transformed the understanding, diagnosis and classification and specialist immunohisto-chemistry, immunophenotyping, cytogenetics and molecular methods have become essential adjuncts to traditional morphology for accurate diagnosis and classification. These techniques are now fundamental for diagnosis, treatment and monitoring in an era of monoclonal antibodies and novel agents targeted for specific molecular abnormalities. Following lymph node or bone marrow biopsy, the bulk of disease remains in the patient at the time of treatment. This is unlike most solid tumours which are initially biopsied and then surgically removed i.e. a double histological assessment. Usually there is no second tissue sample for confirmation of haematological tumours; inaccessibility and limited cells for diagnostic analysis can also be a problem. The maximum diagnostic, therapeutic and prognostic information must be generated by the laboratory on the initial biopsy sample.

2. Accuracy of diagnosis: An accurate diagnosis is the starting point of patient management which should be based on comprehensive diagnostic and prognostic information to define the most appropriate treatment. This is the single most important aspect of improving patient outcomes in haematological cancers. There is historical evidence of significant levels of inaccuracy of diagnosis derived from audits and reviews which demonstrated errors potentially affecting treatment. These are summarized in Table 1.

Table 1. Summary of lymphoma histology audits and reviews adapted from 'Improving Outcomes in Haematological Cancers - The Manual' 2003. (NHL – Non Hodgkin Lymphoma, HL – Hodgkin Lymphoma, REAL- Revised European American Lymphoma)

Review/Audit	Major Findings
1. **All Wales Lymphoma Pathology Review Panel**[10] 2 year central review of 275 lymph nodes (1998-2000)	Major diagnostic discordance in 20% of cases: -5 cases diagnosed as benign were lymphoma. -13 cases diagnosed as lymphoma was benign or a non-haematological malignancy. -15 cases changed from NHL to HL or vice versa. -16 cases of NHL assigned to a different prognostic group. -21% diagnosed as lymphoma but no REAL classification. -17 cases would have had a change in management strategy and first-line treatment was altered in 12.
2. **Lancashire Hospital**[11] Regional Centre Review	-36% had major discrepancies.
3. **NE England audit**[12] 100 lymph nodes	-26% diagnostic discrepancy rate that would have changed management.
4. **Scotland and Newcastle Group**[13] 549 cases of Hodgkin Lymphoma	-28% had revised histological subtype and a resulting change in management in 10%.

Diagnostic errors from trials also confirmed the problem and this is exemplified by the findings of the UK MRC LY10 trial in Burkitt lymphoma (BL)[14]. About 50% of patients entered into the trial were shown not to have true BL on expert review. It is likely that many patients with BL are not accurately differentiated from diffuse large B-cell lymphoma (DLBCL); a crucial starting point of management as BL is very successfully treated with intensive chemotherapy but not with CHOP-R which is standard treatment for DLBCL. This highlights the problem that BL cannot be reliably diagnosed by morphology alone. Although an important starting point, morphology is no longer the diagnostic gold standard and must be complemented by immuno-phenotyping and cytogenetic/ Fluorescent *insitu* hybridization (FISH) investigations.

Early evidence for unacceptably high diagnostic error rates was based on publications and audit data from 1995 to 2003. This has been criticized as being out of date and not reflecting current diagnostic practice and accuracy but in two recent reviews confirm that the underlying problem still remains. The first was an audit carried out in Greater Manchester (A Norton and R Byers 2008; personal communication) which found the serious error rate to be approximately 15%. The second review was undertaken in North London (Table 2) and whilst error rates fell between 2003 and 2008, they are still substantial (13-15%) resulting in minor or major changes or delays in treatment[9].

Table 2. Summary of the outcome of expert review in a large London study of lymphoma diagnoses

Review	Major Findings
North Central London Lymphoma Network 1,949 patient samples were subject to expert review between 2003 and 2008.	• Overall discordance rate of 27.3% identified. Among the 10 most commonly referred lymphoid malignancies, the discordance rate varied between 3.6% and 34.1%. Of the 512 discordant diagnoses, it was possible to assess 350 patients to determine whether expert central review would have altered patient management. • In 39 patients (11%), would have resulted in a significant change to the clinical management of the patient; 19 of these patients (5.4%) were misdiagnosed with either reactive or malignant conditions. • In 136 patients (39%), only minimal change(s) to patient care would have been made after central review. • In 175 patients (50%), the primary diagnosis provided insufficient or outdated information and would have resulted in either delayed or potentially inappropriate treatment. • 42% of samples required additional ancillary tests to confirm or establish the final diagnosis. During the 6-year study, the discordance rate improved significantly, decreasing from 32% to 13%.

These problems are by no means limited to the United Kingdom or solely to lymphomas. Similar problems in lymphoma[15] and acute leukaemia[16] diagnosis were reported from the USA and support the view that expert review of pathology improves diagnostic accuracy.

3. Variable service provision: Services may range from single-handed pathologists with little access to specialist diagnostics through to fully integrated specialist diagnostic laboratories. This variability raises concerns about access to specialist technologies and accuracy of diagnosis. Reduplication, inappropriate testing and contradictions can result if key investigations are carried out in multiple separate laboratories. A fundamental weakness of the traditional approach is that the onus is placed on the clinician to bring together these disparate and sometimes highly complex pieces of information. Facilities are often geographically dispersed with differing levels of expertise and resources. It is not appropriate or possible for all laboratories to provide all specialist diagnostic technologies. Usually, local initial assessment of specimens should be followed by appropriate referral to identified specialist immuno-phenotyping, cytogenetic and molecular genetic diagnostic services with enhanced levels of equipment and expertise.

4. Diagnostic errors and quality assurance: Errors in diagnosis need to be minimized. The traditional approach to the diagnosis of leukaemia and lymphoma can be duplicatory and ineffective. Samples are often sent to multiple laboratories specializing in individual techniques but with no integrated diagnostic pathway. Each laboratory carries out its own series of routine investigations based on initial referral information and an unconfirmed diagnosis. If the referring diagnosis is incorrect, the diagnostic tests undertaken may be irrelevant to the clinical problem. The separate laboratories have no mechanisms for communicating a change to more appropriate tests. Diagnostic effectiveness depends on rational choices of investigative techniques, balancing cross-validation against reduplication.

5. Service planning: Precise and reliable figures for incidence and survival for patients with haematological cancers are required to judge whether clinical outcomes are internationally comparable. Future service planning requires knowledge of incidence and prevalence rates which may change over time[17]. This information is rarely available.

6. Resource implications: There are resource implications for setting up these specialist services which have high capital and revenue costs as well as requiring scientific staff with the necessary laboratory expertise. Multiple new technologies are available for routine use including multicolour flow cytometry, gene expression profiling, single nucleotide polymorphism array karyotyping and high throughput sequencing. Such technologies are capital intensive and a planned approach to the development of integrated diagnostic pathways is necessary rather than an ad hoc approach. Diagnostic centres need sufficient skills and capacity to research, fund, evaluate and implement these new technologies.

The future

If progress is to be made, current institutions and systems must be challenged to improve service organization, operation and delivery. Change is dependent partly on historical services, resources available, geography and political willpower.

Recent developments in classification and technology provide a solution to the problems outlined above. The WHO classification defines each type of leukaemia and lymphoma by clinical characteristics, morphology, immunophenotype, cytogenetic and molecular features. If all of the defining features can be demonstrated, there is a high probability that the diagnosis is correct.

Examination of the evidence above and personal experience of implementation, development and delivery of integrated diagnostic services forms the core of this review which must answer a key question: ***What is required to improve diagnostic services and patient outcomes?*** Irrespective of how the service is structured there are a number of essential components:

1. The Integrated Diagnostic Pathway and Report: The underlying principle is that effective working

requires an integrated diagnostic pathway. The design of the pathway comprises four components: 1) Selection of the most appropriate diagnostic platforms for a particular clinical situation. 2) Selection of investigations for each specimen to provide cross-validation using the WHO principle of multi-parameter disease definitions whilst avoiding unnecessary duplication. 3) Optimal analysis of small specimens with limited tissue or cell availability. 4) The final output of the process should be an integrated report that includes all the information needed for patient management.

2. Commissioning the service: National organizations and commissioners of the service should ensure that providers have the necessary size, structure, organization, laboratories, technologies, integrated operating protocols and information technology (IT) systems. They should ensure that clinical units refer samples to the nominated diagnostic centre and audit compliance of referral patterns. Proof of audits of standard operating procedures, turnaround times and participation in quality assurance schemes help ensure the overall quality of the service.

3. Organization: The service should have clearly defined organizational structures including an identified person responsible for the operation of the service who overseeing the design of diagnostic pathways, use of resources and standards of reporting. The service lead be formally accountable to a single institution for providing the service on behalf of referring organizations and clinicians. Both managerial and financial responsibility should rest with a single organization/hospital with defined business planning processes to facilitate operation, development and co-ordination of diagnostic developments.

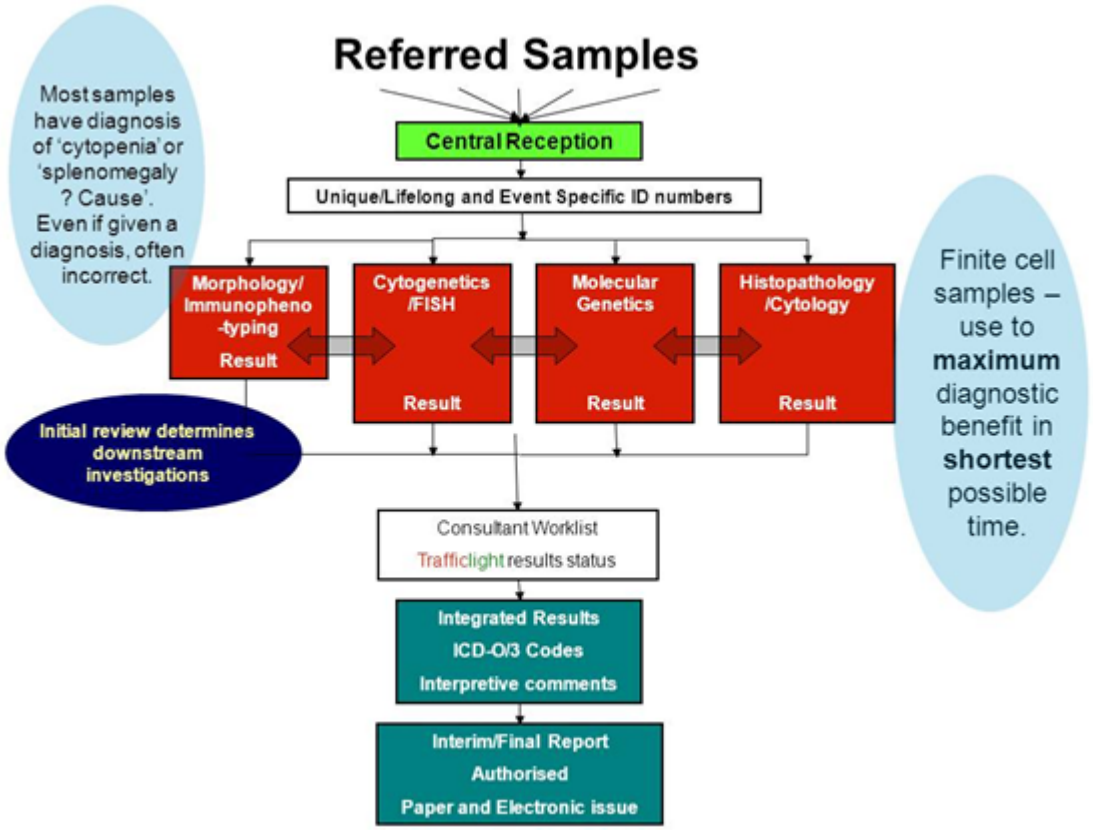
The speed of clinical and diagnostic technological development demands effective horizon scanning, forward planning and funding for implementation of new diagnostic and therapeutic technologies.

4. Laboratory location and integration: The provision of an integrated diagnostic service is most easily achieved in a single institution with co-located laboratories and a full complement of specialist staff and equipment. In many cases this will require significant re-engineering of existing services to achieve the maximum benefits. It is possible, although much more difficult, to design an integrated service based around multiple laboratories in different institutions, each providing a separate component of the service. However, this hinders interdepartmental communication and reduces opportunities for cross-discipline working of staff.

5. Operational processes: A key element of operational quality is achieved through a central reception point for all specimens and by co-locating the component laboratories in a single institution. Even if some tests are performed at different locations there must be a logical flow of samples from registration, through initial screening, systematic investigation, reporting and authorization. There should be standard operating procedures covering sample handling, the diagnostic pathways and compilation of reports.

Integrated diagnostic processes (Figure 1) require a systematic approach to the choice and sequence of tests. It is essential that changes to test requests can be made after a review of initial results, usually cytomorphology and immunophenotyping, so that unexpected or discordant results can be more appropriately investigated. It is common for samples to be sent without a firm diagnosis (e.g. 'pancytopenia – unknown cause' or 'splenomegaly'). In addition, it is not uncommon for samples to be referred with a firm diagnosis but subsequent investigation proves this to be incorrect. Downstream investigations such as cytogenetics require specific cell preparation techniques for optimal analysis; cell culture methodologies differ depending on whether the problem is in the myeloid or lymphoid lineages. FISH and molecular testing require specific probes or primers; if you don't ask the right question you will not get a useful answer!

Figure 1. Integrated diagnostic processes and resulting/reporting pathways.



Results from each laboratory/discipline, released by senior scientific staff, are reviewed and compiled into a fully integrated report by senior clinical/medical staff with appropriate expertise. This affords the opportunity for validation and cross-checking before the report leaves the laboratory. If results are discordant, further investigation may be required. Final diagnoses should be identified by the WHO International Classification of Diseases for Oncologythird revision (ICD-O-3) nomenclature and codes and the final report can then be released to the referring clinician.

6. Information Technology: The essential 'glue' that makes all this possible is an Information Technology (IT) system that records the diagnostic pathway, compiles and issues authorized reports to users. This can be a commercially available or custom-built system. Computer software must be designed to support precise identification of haematological malignancies and be updatable to accommodate changes in WHO classification. IT systems also need to be flexible for changes to diagnostic test repertoires.

To support precise identification of haematological malignancies, laboratories should have computer software systems designed with the WHO diagnoses and ICD-O-3 codes.

7. Diagnostic Pathways and Technologies: A systematic approach to the investigation of suspected leukaemia and lymphoma based around a carefully designed pathway is essential. If this approach is taken then important entities that cannot be reliably identified by morphology alone will not be misdiagnosed. The problem is not simply a function of morphological interpretation, as shown by the European study of the reproducibility of immunocytochemistry[18]. Conditions will only be recognized reliably if a systematic diagnostic pathway is applied to all specimens and this particularly applies to rare, low-frequency tumours.

The key diagnostic technologies are flow cytometry, histo-cytopathology and immunocytochemistry, cytogenetics/fluorescent in-situ hybridization (FISH) and molecular genetics. Techniques need to be regularly reviewed and the need for training and developing specialist staff is a critical consideration.

Multiple new technologies are becoming available. These include advanced 8-10 colour flow cytometry, gene expression profiling, whole or targeted genome copy number analysis and high throughput sequencing. Laboratory services need to be able to accommodate rapid implementation of new technologies, maintain technology research to feed diagnostic development and have capital investment capability. Associated with this is the need for development of staff skills.

8. Final report: An integrated report that includes all information needed for patient management should be available in the shortest possible timescale for clinical discussion and treatment planning. The final report should summarize the results of investigations performed, contain an interpretative comment and a final diagnosis using the terminology of the WHO classification/ICDO-3 coding. It should be possible to review, revise or amend reports if laboratory findings change or add to the initial diagnosis.

9. Interface with Clinical Haemato-oncology: The specialist laboratory must provide support to clinical teams from referring institutions. There should be clearly identifiable contacts for discussion of clinical problems. Modern IT systems offer rapid and secure mechanisms for return of results to clinicians and can be organized to provide secure electronic 'data warehouse' repositories for results look-up.

10. Quality assurance: Quality is assured at several different levels; national service planning, organizational integrity, systematic investigation protocols, independent pathology accreditation and participation in external quality assurance schemes.

Organizational design is fundamental to achieving the ideal management, operation, environment, facilities, equipment and staff. Quality of the organization and diagnostic systems is assured by all component laboratories participating in laboratory accreditation and external quality assurance schemes. Operational consistency is confirmed by effective internal quality assurance including audit trails for samples demonstrating that the diagnostic pathway has been followed.

Systematic testing protocols should define order and choice of test according to the suspected diagnosis or clinical scenario so that the final interpretative report contains the relevant information for management of the patient and avoids possible duplications and contradictions that may arise when these investigations are undertaken in separate laboratories in different organizations.

The final report with diagnosis, prognostic indicators and interpretative comments can then be authorized by a single designated Haemato-oncology pathologist. Finally, the quality of the system is completed by the pathologists working with the clinical teams who are the final quality check in the process, confirming that clinical, imaging and pathology results are all concordant.

11. Training, Research and Development: The host institution should have sufficient resources to undertake research, bio-banking, technology evaluation and service development. Appropriate resources are essential for teaching and training medical and scientific staff on rotation from referring hospitals. Diagnostic training is increasingly delivered in centres and has the advantage of exposing the trainees to the whole spectrum of specialist investigational techniques. Trainees will see a far greater number of specimens and technologies with a greater exposure to rare cases from a larger catchment population.

12. Cost Effectiveness: The cost implications of integrating laboratories vary according to the historical degree of centralization achieved, additional equipment required and the size of population served. There is little evidence about the minimum size of catchment populations but in my experience, the workload from a catchment population of at least 4 million allows robustness

of organization with acceptable cost effectiveness; a higher population number would justify greater investment in automation and streamlining of molecular technologies. To realize savings, obsolete methods of investigation need to be discarded; again emphasizing the need for coordinated development rather than the ad hoc approach that is often exists.

Diagnostic centres need sufficient capital and capacity to fund research, develop, evaluate and implement new technologies in the setting of increasing clinical requirements for diagnostic sub-classification, prognostic factors, targeted treatments (e.g. detection of BCR/ABL mutations in Chronic myeloid leukemia for Tyrosine Kinase resistance and p53 deletion in Chronic lymphocytic Leukaemia) and minimal residual disease detection.

Conclusions

There are very significant quality improvements to be gained from implementing an integrated service model. The central requirement is to demonstrate diagnostic accuracy by following systematic protocols for investigation and reporting. Financial savings are possible from improved efficiency and centralized services as high fixed capital costs are offset by high workload and lower unit test costs. Experience in several centres has shown that services can be provided by a centralized facility serving large catchment populations whilst maintaining a high level of integration with clinical services.

Real progress requires a national approach to achieve uniformity of quality to modern diagnostic technologies and expertise. This is best achieved through the establishment of a national collaborative specification for the service.

Such an approach provides clarity of organization and operation with appropriate capacity, access, equipment and expertise. Improved ascertainment of diagnostic data permits epidemiological studies and service planning. A minimum critical mass of resources is required to maintain research, education and training for the future needs of the service together and strong links to clinical services to enhance patient care.

Authorship

Contribution: This is the sole work of Dr RM Ireland. Conflict-of- interest disclosure: The author declare no conflict of interest

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Reprints: Not available from the author.

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Research paper

Correlation of disease manifestations with laboratory parameters in Leptospirosis

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Key words: blood; leptospirosis; laboratory; renal.

Abstract

Background: Leptospirosis can present as outbreaks in many countries contributing to significant morbidity in the affected populations. Studies published on this infection have focused on the prevalence of the disease, molecular diversity of the pathogen and disease complications. There is a substantial lack of publications on the basic laboratory parameters during the course of the disease and their correlation with disease progression.

Objective: To find the correlation of clinico-laboratory parameters with the disease process.

Methods: In a cohort of 48 patients clinically diagnosed and confirmed by serology to have leptospirosis and managed in the medical wards of Provincial General Hospital Ratnapura from January to June 2011, disease course and the routine biochemical and haematological test parameters were studied. The correlation of the test results to the course of the disease and its complications was calculated.

Results: The clinical manifestations represented the well-known symptoms of fever (95%) and myalgia (77%) but jaundice was not common (21%). Bleeding was rare (14%) while neurological complications were extremely rare (6%). Leukocytosis, described as common in this condition, was not observed in majority (66%) of the patients. Thrombocytopenia was common (73%) although very rarely severe. Renal and liver function abnormalities were noted in a good proportion of patients (79%). A significant positive correlation (Spearman's rho = 0.414, P<0.01) was observed between thrombocytopenia and renal impairment.

Conclusions: Clinical and laboratory pattern of the patients in the study did not exhibit all the features classically described for leptopsiprosis. Importantly, platelet count was identified to be a possible marker for development of renal impairment.

Introduction

Leptospirosis is a zoonotic disease of global importance. It is considered a re-emerging infection due to recent large outbreaks in developing countries following floods[1]. This disease has a worldwide distribution but is more prevalent in the tropics where the conditions for disease transmission are more favourable[2,3]. However, individuals from developed nations are also at risk for the infection from increasing international travel and greater participation in outdoor recreational activities such as freshwater swimming, kayaking and hiking[4]. Recent epidemics have occurred in Sri Lanka with a call for increasing diagnostic capabilities[5]. Although leptospirosis is no more considered as primarily an occupational disease, in the area where this study was conducted, the higher incidence of the disease may be attributed to its main industry, gem mining.

Methods

This retrospective study was carried out at the Provincial General Hospital Ratnapura from 2011 January to 2011 June. Patients clinically diagnosed as leptospirosis and confirmed by detection of leptospira IgM by ELISA during this period, were included in this study. A study protocol was predesigned and the required data were extracted

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from patients' hospital notes. Data were collected by a Medical Officer on the clinical manifestations of the disease the patients had, with an emphasis on the time of occurrence of these symptoms and the clinical signs. The laboratory parameters studied included haemoglobin concentration, white cell and platelet counts, coagulation times, renal and liver function parameters, (S. Creatinine, Blood urea, SGOT, SGPT, S. Bilirubin) erythrocyte sedimentation rate and urinalysis. The treatments given to the patient, development of complications and duration of stay were also recorded.

Results

A total of 48 patents records were analyzed in this study over a period of six months. The age range was 15 to 63 years.

Clinical features: The symptoms and signs they had at presentation and during the hospital stay are given in table 1. Fever (95%) and myalgia (77%) were the commonest symptoms. Headache, nausea and vomiting were seen in around half of the patients. Bleeding was noted in 7 patients. Bleeding manifestations observed were ear, nose and throat bleeding, haematuria, gum bleeding, malaena and haematemesis. Although conjunctival suffusion was identified in just over one-third of the patients, jaundice was not a common symptom and was clinically only noticeable in about one-fifth of the patients. Neurological manifestations were rare (6%) and included drowsiness, neck stiffness and flapping tremor.

Treatment: All the patients were treated with penicillin (25%) or cefotaxime (70%) except one who received doxycycline and another levofloxacin.

Laboratory parameters: Sixteen out of forty eight (33.3%) patients had white blood cell count above normal (greater than 11.0 x 10^9/l) at any given time during the illness. In the rest it was normal or low during the entire hospital stay. In 5 (10%), the white count was below normal (less than 4.0 x 10^9/l) at least once during the course of the illness. In 35 (73%) of the patients, the platelet count dropped below 150 x 10^9/l during the hospital stay. In 15 of these cases, it went below 50x 109/l and in 9; it dropped below 20x10^9/l. ESR had been done only in 9 patients and of those 5 (56%) were above 100mm/1 hour. Prothrombin time was done in 18 patients and prolongation was seen in 5 (28%). Activated partial thromboplastin time was performed in 12 and prolongation noted in 3 (25%) patients only. Blood urea and/or serum creatinine were increased in 30 (62%) patients. Elevation of liver enzymes appeared to be universal although clinically jaundice was not observed in many of these patients. In 44 (96%) patients, serum glutamic pyruvic transaminase (SGPT) was found to be high. Serum bilirubin was done in only 22 patients and it was elevated in all except 3 patients (86%). It did not increase in conjunction with rest of the liver enzymes at all times but very high values were observed in some patients (> 500µmol/l in 5 patients). Considering urinalysis, all the patients except four (94%) had albuminuria. Red cell casts were seen in 31(65%). Thirteen had significant number of pus cells (greater than 10 per high power filed) in the urine. Fourteen patients had complications of the disease. Four (8%) had myocarditis, 8 (16%) developed acute renal failure and 2 (4%) had pneumonia and pleural effusion. There were no deaths in this cohort of patients.

Clinico-laboratory correlation: A significant positive correlation was seen between thrombocytopaenia during the disease course and the renal impairment (Spearman's r = 0.414, P<0.01). However there was no significant association between the overall development of complications and decrease in platelet count (Spearman's r = 0.192).Eleven(11) patients with thrombocytopenia developed either myocarditis, renal failure with oliguria or pleural effusion, while in the patients who did not experience a thrombocytopenia only two had complications of the disease which was also pyelonehritis and chest infection.The duration of stay ranged from 3 to 16 days with a mean of 6.8 days. Development of thrombocytopaenia and renal impairment did not show any effect on the length of stay in hospital. Bleeding manifestations such as nasal bleeding, gum bleeding and haematemesis occurred, not unexpectedly, when the platelet count fell below 20 x 10^9 /l. However there was no significant correlation between conjunctival suffusion and low platelet count (Spearmans's r = 0.069, p = 0.6)

Table 1. Symptoms and signs of patients diagnosed with leptospirosis

Symptoms and Signs	Number of patients	Percentage (%)
Fever	46	95
Myalgia	37	77
Nausea and vomiting	25	52
Headache	24	50
Conjunctival suffusion	18	37
Jaundice	10	21
Lung symptoms	9	18
Bleeding manifestations	7	14
Neurological symptoms	3	6

Discussion

Accurate diagnosis of leptospirosis is a challenge to the clinician, especially where the diagnostic facilities are limited. Due to its similarity in presentation to that of other arboviruses (Eg: dengue fever), this condition may be overlooked unless a high degree of suspicion is maintained. Secondly, confirmatory tests for this infection may not be easily available in the endemic areas and even if accessible can take time for accurate diagnosis to be made. Thirdly, early prediction of complications may be useful for the decision of intensive monitoring. Therefore, understanding the changes in basic laboratory parameters available would be worthwhile in making a provisional diagnosis as well as predicting the disease course.

In this study, we identified that fever, myalgia and liver enzyme abnormalities are almost universal in most patients with leptospirosis. Characteristically, calf or lower back myalgia and conjunctival suffusion without purulent discharge are considered highly suspicious of leptospirosis[4] although in this study, conjunctival symptoms were not common. One of the useful symptoms which would point against the diagnosis of leptospirosis is neurological disturbance especially consciousness disturbance which can often be observed in the other diagnoses which mimic Weils disease. In typical cases of leptospirosis, jaundice usually appears after several days of illness and is not often associated with hepatocyte damage but appears to be related more to the cholestasis of sepsis. In our patients, although liver abnormalities were common, jaundice was rare reflecting this picture.

Classically, the differential diagnosis of leptospirosis from dengue fever has been based on certain clinical and laboratory findings[6]. One of the key features mentioned is a lower white cell count with dengue and leukocytosis in leptospirosis. In this study, we identified in almost two-thirds of patients, white cell count had not risen anytime during the hospital stay. A summary of similarities between clinical presentations of dengue and leptospirosis and pointers towards the latter diagnosis is given in table 2.

One of the interesting findings of our study has been the correlation of laboratory parameters to the clinical outcome and we noticed that patients with severe thrombocytopenia tend to develop complications like renal impairment and myocarditis. It may be hypothesized that platelet aggregation causing microthrombi in the different vasculature may have contributed to the organ

Table 2. Differentiating features of leptospirosis from dengue fever

Features common to both disease	Features favouring leptospirosis
• Conjunctival suffusion	• Less petechiae
• Joint/bone pains	• Less frequent neurological abnormalities
• Rash	• Abnormalities in urine analysis
• Decrease in white cell count	• Less petechiae
• Severe thrombocytopenia	
• Abnormalities in liver function tests	
• Conjunctival suffusion	

injury and also thrombocytopenia. Although, the drop in platelet count may simply reflect disease severity, we would consider regular monitoring of a simple blood test like the complete blood count and be fastidious in the clinical management of the patients who are noticed to have decreasing platelet counts. It is also important to have noted no correlation with bleeding and the degree of thrombocytopenia suggesting lower platelet counts are not relevant in the bleeding manifestations of this condition. Bleeding may have been caused by abnormal coagulation function or fibrinolysis although these were not studied in this population due to limited resources.

In this study some of the classical laboratory and clinical features of Leptospirasis were not seen commonly. More importantly a clinico-laboratory correlation between the platelet count and renal impairment was observed. However further studies on a larger cohort of patients is required to make definitive inference on these findings.

Authorship

Contribution: NS and JT conceived the idea, prepared the proforma, analyzed the results and prepared the manuscript. KJ, SN and IL managed the patients and provided useful comments during preparation of the manuscript. IL collected the data. All authors approved the final version.

Conflict-of-interest disclosure: The authors declare no conflict of interest.

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Case report

A case of dengue haemorrhagic fever (DHF) successfully treated with prothrombin concentrate complex at Lady Ridgeway Hospital (LRH) for Children, Colombo – ? A historic landmark

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Abstract

Dengue Haemorrhagic Fever (DHF) has become a major public health concern in Sri Lanka. Shock and significant bleeding are the more commonly seen dreaded complications of this disease and remains a major challenge for all clinicians. Using blood products such as fresh frozen plasma (FFP) and cryoprecipitate is controversial and is not advocated by many mainly because of the fact that these fluids too, like the patient's plasma, leak out of the leaky capillaries and the significant volumes needed to correct coagulation abnormalities may add to the volume overload. The patient described in this case, who had major bleeding manifestations, was successfully treated with a single vial of Prothrombin Complex Concentrate (PCC). To the best of our knowledge, this is the first time that PCC has been used to treat bleeding manifestations in DHF although the more expensive Factor VIIa has been used before with varying results. The promising results seen in this case may open a new pathway towards successful management of bleeding manifestations in DHF which is far less expensive than factor VIIa and does not lead to fluid overload.

Case report

A 12 year old girl presented with fever, vomiting and abdominal pain for 3 days. She had no bleeding manifestations and had not received Non-Steroidal Anti-inflammatory Drugs (NSAIDs). There was no past history of bleeding tendencies or any other significant illnesses apart from a mild intermittent asthma. She was looked after by her father and two elder sisters as her mother was abroad.

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On examination, she was febrile and mildly dehydrated. There was no pallor or evidence of bleeding into the skin. Her cardiovascular system was normal. Breath sounds were reduced in the right lower zone and a dull percussion note was audible in this area indicating the presence of a pleural effusion. Generalized abdominal tenderness was found without guarding and there was no evidence of ascites.

On admission her full blood count was as follows. WBC – 6,800/mm^3 (N- 78%, L-20%); Haemoglobin (Hb) level – 14.6 g/dl; PCV – 43.8%; Platelet count – 91,000/mm^3

On the fourth day of illness, she was ill and continued to leak. Her haemodynamics were stable throughout the entire critical phase.

On day 5 she showed clinical evidence of bleeding for the first time with the development of mild gum bleeding and haematuria. The following day (day 6 of illness) she had haematemesis and malena. An naso-gastric (NG) tube was kept in situ to monitor for fresh bleeding and she continued to fresh blood aspirates on day 7. The bleeding tendency worsened on this day with evidence of puncture site bleeding.

Her coagulation profile was abnormal from day 4 onwards before clinical detection of bleeding. Despite being transfused FFP and Cryoprecipitate daily and platelets twice, the APTT and PT/INR continued to rise. PT/INR increased from 17.2/1.7 to 20.8/2.3 from day 4 to day 7 and the APTT from 36.5 (28.6) to 65 (28.6). On day 7 the thrombin time (TT) was elevated more than 2 minutes.

Her haemoglobin dropped from 13.3 g/dl to 8.9 g/dl and PCV from 39.6% to 26.8%. She received three blood transfusions on day 6 and 7.

On day 7 as bleeding persisted with significant abnormalities in PT/INR/APTT and TT one vial of PCC was transfused. Soon after PCC transfusion, coagulation studies showed a significant improvement. (PT/INR dropped from 20.8 / 2.3 to 13.1 / 1.15; APTT from 65 to 44)

She was transferred to the Intensive Care Unit after the PCC transfusion, for close monitoring. She was noted to have fresh NG bleeding and puncture site bleeding during that day, but this settled gradually on the same day and no bleeding was noted thereafter.

She recovered steadily and all her abnormal coagulation test values gradually returned to normal. On day 8 the TT continued to be elevated when the PT/INR and APTT showed significant improvements (PT/INR - 15.1/1.37; APTT - 38.1; TT - more than 2 minutes). The D-Dimers were significantly elevated at 15.6 mg/l (< 1 mg/l).

On discharge from hospital (on day 12 since the onset of the illness), her Hb level was 10.6 g/dl; PT/INR 13.2/1.1; APTT 26.6 (28.6); and TT 13.7 (12.4). Both the PT/INR and APTT returned to normal earlier than TT, which returned to the baseline slower.

A summary of the investigations are given in Table 1.

Discussion

Bleeding, both overt and concealed, is a major clinical problem in DHF, and predicting and treating it remains a major challenge for clinicians.

Many studies have been done to find early predictors of bleeding. Various parameters have been evaluated with varying results and there is no consensus on any such parameter to date. An area of interest for many of these studies has been the derangement of coagulation studies[1-5]. This patient's INR and APTT were both abnormal by day 4 of the illness, long before bleeding manifestations were clinically detectable. They continued to rise despite being transfused with FFP, cryoprecipitate and platelet concentrates regularly indicating the questionable benefit of these blood products in the management of DHF.

There is evidence that both the coagulation and fibrinolytic pathways are abnormal in DHF[1]. Her persistently elevated TT on day 8 of illness, at a time when INR and APTT were returning to normal, indicates that the fibrinolytic pathway had remained abnormally active for a longer duration than the clotting abnormalities. However, Fibrinogen Degradation Products (FDP) and Tissue Plasminogen Activator (tPA) levels could not be done for confirmation. Anti-fibrinolytic agents may be a useful form of treatment for bleeding in DHF

Table 1. Summary of Investigations

	Day 4	Day 7 Before PCC	Day 7 After PCC	Day 8	On Discharge
INR	1.7	2.3	1.15	1.37	1.1
APTT(sec)	36.5	65	44	38.1	26.6
TT(sec)	-	> 120	-	> 120	13.7
ALT(IU/L)	80	74	85	145	-
AST(IU/L)	196	425	426	583	-

where there are no contraindications for its use but more studies are needed to confirm this.

The use of FFP and cryoprecipitate in DHF remains a controversial topic, as mentioned earlier. Some believe that it is of no use practically as the significant volume of FFP needed to correct coagulation abnormalities would add to the volume load. The strict fluid management in DHF does not permit such high volumes to be transferred to patients who are in the critical or early recovery phase. There is a strong possibility that the patient would get overloaded if such high volumes are transfused. However, some clinicians believe that there is a significant benefit in these blood products and routinely use them for patients with DHF and coagulation abnormalities. In this patient, although several packs of FFP and Cryoprecipitate were transfused, the INR and APTT continued to rise and bleeding persisted.

The transfusion of Prothrombin Complex Concentrate (PCC) significantly improved the coagulation abnormalities and there was a remarkable reduction in bleeding. A single dose which contained 25 units/kg (of factor IX) showed satisfactory results and its efficacy was evident within a few hours as the bleeding manifestations improved dramatically. The volume that was transfused was very small and was not significant enough to cause fluid overload.

Although factor VIIa has been used in severe bleeding manifestations in DHF, the product's extremely high cost and inconsistent availability are well known. It has been used in our country for some time but there is a lack of consistent scientific data on its efficacy. Prothrombin complex concentrate is significantly less expensive and widely available. If PCC can be used in place of VIIa to arrest bleeding in DHF it would be extremely beneficial and cost effective considering the cost differencel of these two products. However, more comparative studies are needed to draw conclusions.

To the best of our knowledge this is the first time PCC has been used to treat bleeding manifestations in DHF at LRH. The promising result achieved with this patient has given hope for a better future in dengue management. We believe that there should be clinical trials in Sri Lanka to determine the efficacy of PCC and if positive results are achieved it would make a remarkable difference for patients with bleeding manifestations in dengue.

In this landmark achievement we greatly appreciate the dedicated services of all staff members who contributed towards saving this patient's life.

Authorship

Contribution: All authors have contributed equally. All authors approved the final version.

Conflict-of-interest disclosure: The authors declare no conflict of interest

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Case report

Venous thrombosis at unusual sites – three case reports

C S Moonesinghe[1], **H W C K Kulathilake**[2]

Introduction

The most common manifestations of venous thrombosis are deep vein thrombosis of the lower limbs and pulmonary embolism. Venous thrombosis at other 'unusual sites' is well documented but with paucity of randomized trials of standard anticoagulation therapy. Here, we report three cases, one case of cerebral sinus thrombosis and two cases of portal venous thrombosis with different clinical manifestations.

Case 01:

A 28 year old female, on oral contraceptive pills (OCP) presented with gradually worsening headache and numbness of the right hand for two weeks. MRI of the brain revealed superior sagital sinus thrombosis (Figure 1). Patient was anticoagulated with enoxaparin (therapeutic dose) 1 mg /lg/ twice a day for 05 days followed by warfarin. Patient's symptoms gradually improved. Dilute Russel's Viper Venom Time (DRVVT) screening and Ham test were negative and warfarin was discontinued after one year. Patient was advised to stop oral contraceptive pills as it was a risk factor for cerebral sinus thrombosis.

Case 02:

A 40 year old male presented with persistent abdominal pain for 3 days duration. CT scan showed portal vein thrombosis (PVT) with extensive oesophageal varices and moderate splenomegaly. There was no evidence of cirrhosis and cavernous transformation. As the patient was symptomatic he was anticoagulated with unfractionated heparin followed by warfarin. Banding was done for oesophageal varices. Warfarin was continued for 06 months. Repeat CT scan of the abdomen showed persistent thrombus with cavernous transformation. DRVVT, heritable thrombophilia screening (factor v (F5) Leiden, MTHFR 677C>T mutation and prothrombin (F2) 20210h>A gene mutation) and Ham test were negative. *JAK2 V617F* mutation was not detected.

Case 03:

A 34 year old male presented with an episode of haematemesis. Upper GI endoscopy revealed grade III oesophageal varices. CT scan abdomen showed left portal vein thrombosis with cavernous transformation and splenomegaly with no evidence of cirrhosis. As this was an incidental finding patient was not anticoagulated. Banding was done for oesophageal varices. Above investigations (case 2) including *JAK2 V617F* mutation were negative.

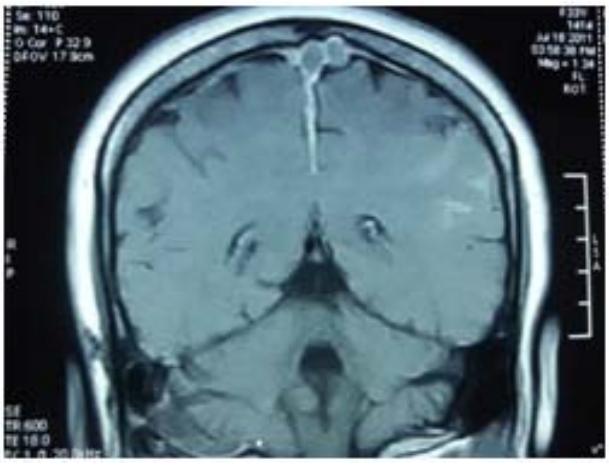

Figure 1. Superior saggital sinus thrombosis in MRI.

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Discussion

Cerebral venous and sinus thrombosis (CVST) should be considered in young and middle-aged patients with recent unusual headache, stroke-like symptoms in the absence of usual risk factors, intracranial hypertension or haemorrhagic cerebral infarcts. Recognized underlying causes include infection, particularly of the head and neck, systemic inflammatory disorders, leukaemia (especially with asparaginase treatment), head injury and dehydration. While myeloproliferative neoplasms (MPNs), such as polycythaemia vera and essential thrombocythaemia, are causes of CVST, in the absence of overt MPN the *JAK2* mutation has not been associated with CVST. Use of oestrogen containing combined oral contraceptives (C-OCPs) is a known precipitating factor[1].

As early treatment is likely to be safe and potentially beneficial it is recommended that patients with CVST without contraindications to anticoagulant therapy should be treated with therapeutic doses of heparin[1].

The optimal duration of anticoagulation is unknown. Expert consensus recommended initial 07 days heparin treatment followed by a vitamin K antagonist for a minimum of 03 months if CVST was secondary to a transient risk factor, for 6-12 months in patients with unprovoked CVST and in those with 'mild' thrombophilia, such as heterozygous F5 Leiden or F2 G20210A mutation. Long term anticoagulation has been suggested for patients with recurrent episodes of CVST, persistent risk factors (e.g. APLS) and unprovoked CVST. Testing for heritable thrombophilia has uncertain predictive value for recurrence of CVST. Acquired risks should be removed or minimized to prevent recurrence, e.g. C-OCP, hormone replacement therapy (HRT) and obesity.

The most common underlying aetiology of portal vein thrombosis (PVT) is cirrhosis. Other causes are intra-abdominal infection, inflammation or malignancy and blunt trauma or surgery[1,2]. MPNs account for up to a quarter of cases and PVT is a common presenting manifestation of MPN. Therefore all PVT patients should be assessed for the *JAK2 V617F* mutation[1]. Paroxysmal nocturnal haemoglobinuria (PNH) is an important cause of intraabdominal thrombosis. Heritable thrombophilias are an aetiological factor as well as antiphospholipid antibodies.

Acute and chronic portal vein thrombosis may be symptomatic (abdominal pain, nausea), but many episodes are detected incidentally in imaging studies. Patients with chronic PVT usually have symptoms of portal hypertension (splenomegaly, and oesophageal varices).

Current literature recommends anticoagulation in symptomatic portal vein thrombosis[2]. Efficacy of anticoagulation in other forms of symptomatic venous thrombosis provides indirect evidence for anticoagulation of patients with symptomatic portal vein thrombosis. In a patient with an incidental finding of PVT factors that encourage anticoagulation include lack of cavernous transformation, extensive thrombosis that appears to be acute and progression of thrombosis[2]. When chronic portal vein thrombosis is an incidental finding especially in the context of cavernous transformation, usefulness of anticoagulation is doubted[1,2].

However, as the bleeding risk is high due to cirrhosis, oesophageal varices (secondary to portal hypertension) and thrombocytopenia (secondary to hypersplenism) treatment decision has to be taken on individual basis[1,2]. There is no evidence to guide the duration of anticoagulation in PVT. Extended therapy is currently considered in patients with persistent risk factors (e.g. MPN, PNH) and low bleeding risk[1,2].

Both patients (2 and 3) had chronic portal vein thrombosis with evidence of portal hypertension. Considering the symptomatic presentation and lack of cavernous transformation anticoagulant therapy was offered to the first patient. As it was an incidental finding in the second person anticoagulation was not indicated.

Authorship

Contribution: CK collected the clinical data. Manuscript preparation was done by CSM. Both authors approved the final version.

Conflict-of-interest disclosure: The authors declare no conflict of interest.

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CME Article (Series II)

Theme: The clinical significance of the new parameters of the reticulocyte

I Wijesiriwardena[1]

Try this **extended matching question** to assess your knowledge on the new parameters of the reticulocyte.

Which specific reticulocyte parameter is the best to find a diagnosis, a clue to the diagnosis or treatment response, in the given clinical event, 1, 2 and 3.

Each option (a to f) may be used once, more than once or not at all.

Match the best option and write in the space given, next to the question number in the answer grid.

a) Reticulocyte count
b) Corrected Reticulocyte count (reticulocyte index)
c) Reticulocyte production index (RPI)
d) Immature reticulocyte fraction (IRF)
e) Reticulocyte haemoglobin content (CHr)
f) Peripheral blood picture of polychromatic cells

1) A 35 yr old female,POA 32 wks. Hb of 6.5 g/dl. Not on haematinics.
2) A 25 year old male with an Hb of 6g/dl one week after allogeneic bone marrow transplant for acute leukaemia
3) A 60 yr old male on haemodialysis for end stage renal failure, Hb 6.8 g/dl, on 10,000units recombinant erythropoietin (rEpo) thrice a week, with minimal response. Absolute reticulocyte count is normal.

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If you need help to refresh your memory of the definitions please note the following:

1) **Reticulocyte count:** The reticulocyte count is used to estimate the degree of effective erythropoiesis[1],which can be further emphasized as absolute reticulocyte count or as a corrected reticulocyte count or percentage. If anaemia is present, the reticulocyte count is spuriously high and may not reflect true bone marrow responses to anaemia.

2) **Absolute reticulocyte count**: The red blood cell count is used to correct for the anaemia.

3) **The corrected reticulocyte count**: Is calculated using the formula below[1,2]. Reticulocyte count is corrected to the degree of anaemia with the haematocrit.

   Corrected reticulocyte count = reticulocyte count x [actual haematocrit/normal haematocrit (usually 45)].

4) **Reticulocyte production index** (RPI): Which is corrected or adjusted for both premature release of reticulocytes from the bone marrow and the degree of anemia, is another parameter that provides an assessment for adequate bone marrow response to anaemia.

   The reticulocyte production index, is calculated using the formula below[1,2].

   Reticulocyte production index = corrected reticulocyte count/maturation time in peripheral blood in days (correction factor)*

   *To give an indication of the efficiency of the bone marrow, the reticulocyte count is corrected by this haematocrit-dependent factor. Haematocrit 45%, Reticulocyte duration in blood 1 day; Haematocrit 35%, Reticulocyte duration in blood 1.5 days; Haematocrit 25%, Reticulocyte duration in blood 2 days; Haematocrit 15%, Reticulocyte duration in blood 2.5 days.

5) **Immature reticulocyte fraction** (IRF)**:** Immature reticulocyte fraction (IRF) is defined as the least mature fraction of reticulocytes and serves as a mean of assessing reticulocyte fraction[3]. The maturity of reticulocytes is classified based on the amount of stained RNA content by automated machine using fluorescence. The most immature reticulocytes, produced when erythropoietin levels are high, contain more RNA content and fluoresce more strongly than the more mature ones normally present in the peripheral blood[3,8]. IRF can be used to help classify the cause of anaemia, to assess for effective erythropoiesis, and to evaluate for marrow recovery[8,4]. An increase in reticulocytes with the highest RNA content (increased IRF), therefore, reflects early marrow recovery from the conditioning regiments of stem cell transplantation, cancer chemotherapy, or treatment for nutritional anaemias, which usually precedes the increase in absolute reticulocyte count. IRF has also been used to evaluate ineffective erythropoiesis and to differentiate megaloblastic anaemia or myelodysplasia (increased IRF) from other causes.[4,7].

6) **Reticulocyte-haemoglobin content (CHr):** The mean reticulocyte hemoglobin content (CHr) is a parameter that indicates the amount of haemoglobin available to the reticulocytes within the previous 3-4 days. This directly correlates with the functional availability of iron in the bone marrow[12, 9].

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Answer Grid:

Question	Answer
1	
2	
3	

CME Points: 05 (Please send in your answer in the CPD portfolio).

The answers are given in page 35 with the explanations.

PROF. S. N. WICKREMASINGHE ORATION 2013

Erythropoietin. Wonder drug or drug to wonder at?

T J Littlewood[1]

Introduction

Erythropoietin is a 166 amino acid glycoprotein manufactured in renal peri-tubular interstitial cells. The existence of a red cell stimulating protein was first suspected in the 19th century but definite evidence awaited the observation by Erslev[1]. In his brilliantly simple experiments he showed that normal rabbits, injected with plasma from very anaemic rabbits, developed a rapid erythropoietic response. The kidney was found to be the major source of this protein (erythropoietic stimulating factor) and in 1977 erythropoietin was purified[2]. In 1983 the erythropoietin gene was cloned[3].

Erythropoietin in patients with chronic kidney disease

Anaemia, primarily caused by inadequate production of erythropoietin, is very common in patients with chronic kidney disease. In the years after the introduction of dialysis, but before recombinant human erythropoietin became available, anaemia was the single most important problem for patients with dialysis dependent renal failure. Nephrologists of that era became adept at trying to maintain the haemoglobin by paying attention to optimizing dialysis, providing appropriate haematinic replacement, minimizing blood sampling and rationing blood transfusions in an attempt to maintain endogenous serum erythropoietin levels. Despite these measures, most patients struggled to maintain a haemoglobin concentration of more than 7.0-8.0g/dl and around 10% were entirely transfusion dependent[4]. Patients' quality of life was markedly impaired and a focused research effort into the causes and possible treatment for the anaemia was launched. Dr Joseph Eschbach, in a landmark paper in 1967, identified that the core need was to identify a source of erythropoietin which could be administered to these patients[5]. In 1986 Winearls et al[6] published a report on 10 patients with end stage renal failure treated with recombinant erythropoietin (Erythropoiesis stimulating agents-ESAs) administered thrice weekly by intra-venous bolus. All 10 patients responded (haemoglobin increase of >2.0g/dl) of whom nine said that the treatment had made them feel better. This pilot study was rapidly followed by several multi-centre trials which confirmed that a ESA dose of around 200 iu/kg/week would increase the haemoglobin concentration to between 10.0-12.0.g/dl in over 90% of patients and improve the patients' quality of life[7,8].

Common causes of a lack of response or a lost response after initially successful treatment included iron deficiency, co-existent infections or other inflammatory disorders, blood loss and an inadequate ESA dose.

Rarer explanations for unresponsiveness to ESAs are aluminium overload and secondary hyperparathyroidism and occasional patients will be found to have an unrelated problem (such as a primary bone marrow disorder) blunting the erythroid response[9].

Excitement about the benefits of ESAs was tempered somewhat by side effects. Hypertension occurred in about one third of patients, some of whom became encephalopathic, and there were a worrying number of patients with thrombosis of venous access sites. Greater experience in the use of ESAs led to patients having a gentler rise in haemoglobin with fewer thromboses and a lower incidence of hypertension which is treated promptly when it occurs[10].

In 2002 Casadevall reported that 13 patients treated with ESAs for dialysis dependent renal failure developed pure red cell aplasia caused by anti-erythropoietin antibodies[11].

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This complication has been reported in approximately 200 patients world wide with a median time to onset after commencing ESAs of around 9 months. Reassuringly most patients who have the ESA stopped and are treated with immunosuppressive drugs will make a full recovery. The data also strongly suggest that this complication is product specific. Nearly all patients had been treated in Europe with epoetin alfa (eprex) given subcutaneously rather than intra-venously.

Recent studies have emphasised that the target haemoglobin concentration should be around 10.0-11.0g/dl as there is an increase in morbidity and mortality above this level[12, 13].

In his pilot study published in 1986[6], Winearls wrote that he considered the correction of anaemia in renal failure by ESAs to be 'of major importance'. Despite some hiccoughs, he was correct.

Erythropoietin in anaemic patients with malignant disease

Anaemia is very common in patients with malignancies; when defined as a haemoglobin of <12.0g/dl around 40% of patients with cancer will be anaemic at diagnosis and this increases to an incidence of around 75% in patients who receive chemotherapy[14].

There are many possible explanations for the anaemia but the most frequent causes are the cytokine driven anaemia of chronic disease (AOCD) compounded by the myelotoxic impact of chemotherapy[15]. These cytokines, interleukin-1, gamma interferon and tumour necrosis factor, suppress erythropoiesis both directly by inhibiting erythropoiesis and indirectly by inhibiting erythropoietin production in the kidney. IL-6 released by inflammatory cytokines increases the synthesis of hepcidin, a hepatic peptide. Hepcidin blocks iron absorption in the gut and iron release from tissue macrophages. This functional iron deficiency is another important contributor to anaemia in patients with AOCD[16].

Fatigue is a common symptom in patients with cancer and its significance may be underestimated by doctors[17].

The frequency with which fatigue occurs and its importance were emphasised by a survey of nearly 400 patients with cancer reported by Dr. Gregory Curt[18]. 75% of the patients reported that fatigue was their most important symptom and of these 60% indicated that the fatigue had a greater impact on their daily lives and 54% that it lasted for a longer period of time than these other important symptoms of depression, nausea and pain. Fatigue may be caused by both physiological and psychological factors and it seems likely that anaemia is just one of the potential physiological explanations.

The commonest approach to anaemia management in patients with cancer is to ignore it which has the merits of being cheap and simple but not necessarily being in the patients' best interests.

Alternative approaches are to transfuse with blood or to use an ESA.

A summary of 17 trials of ESAs in anaemic patients with myelodysplastic syndrome was published by Hellstrom Lindberg[19]. Of 206 patients just 16% responded with a haemoglobin rise of > 1.5g/dl or the abolition of transfusion need. Patients with sideroblastic anaemia seemed to fare particularly badly with a response rate of 7%.

The combination of ESAs and Granulocyte Colony Stimulating Factor (G-CSF) was reported to increase the response rate and this observation has been supported by a number of investigators[20,21]. In these trials the haemoglobin response to the combination of ESAs/G-CSF doubled or trebled compared to patients treated with ESAs alone.

The response rate in patients with non-myeloid malignancies is rather better than in patients with myelodysplasia. Some of the important trials in this field are shown in table 1[22,23,24,25,26]. Despite design and methodological differences the investigators were able to show that, irrespective of the type of ESA used, the haemoglobin response rate (defined as a Hb rise of > 2.0g/dl) varied between 60-72%. The rise in haemoglobin translated into a reduction in transfusion need and an improvement in the patients' quality of life. These and other studies have also shown that the haematological response to ESAs is the same whether the patient has a haematological tumour or a solid malignancy and irrespective of whether treatment has been with platinum or non platinum containing regimens.

Table 1. Randomized trials of ESAs in anaemic patients with non-myeloid malignancies

Author/Year	Patients	ESAs	Hb	Trans. Need	QOL
Cazzola/1995	146	Epoetin beta	61%	⇩	NA
Gabrilove/2001	3012	Epoetin alfa	68%	⇩	⇧
Littlewood/2001	375	Epoetin alfa	70%	⇩	⇧
Hedenus/2003	344	Darbepoietin	60%	⇩	⇧
Cazzola/2003	241	Epoetin beta	72%	⇩	⇧

Combining ESAs with intravenous iron in randomised trials has improved the response rate with no severe adverse safety events[27].

In the trial by Littlewood et al[24] there was a suggestion that survival was longer in the ESA treated patients compared to those receiving placebo.

However, some studies since have suggested that treatment with erythropoietin can shorten rather than prolong life. A large analysis of over 15000 patients entered into clinical trials of ESAs by Glaspy et al[28] suggested a significant increase in the risk of venous thrombo-embolic disease but no significant increase in mortality in chemo-therapy treated patients. International guidelines, such as those from the American Society of Hematology[29], suggest that treatment with ESAs should be only to maintain a haemoglobin concentration at a level just sufficient to avoid blood transfusion.

Other medical uses of erythropoietin

Anaemia is common in patients with heart failure and is an adverse prognostic factor. In small studies, correcting the anaemia with ESAs improves the patients' exercise tolerance and improves the left ventricular ejection fraction but a recent large study[30] has not shown benefit from ESAs in patients with heart failure although the design of this study (with a target haemoglobin of 13.0g/dl) can be criticised.

A dramatic impact of ESAs as a neuroprotective agent was shown in rats exposed to traumatic or ischaemic brain injury[31]. A trial in a small number of humans has indicated that ESAs are safe and effective after acute stroke[32] but these data have not yet been confirmed.

Conclusions

Erythropoisis stimulating agents are a tried, tested and effective treatment for anaemic patients with both chronic kidney disease and malignant disease. Hard lessons have been learned about their safety but when used appropriately, within guidelines, ESAs are usually effective and safe.

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Audit

An audit of fresh frozen plasma usage in a tertiary care hospital in Punjab

A Sharma[1], **K Bhardwaj**[2], **R Bassi**[3], **P Singal**[4], **H S Bhardwaj**[5]

Key words: fresh frozen plasma, transfusion guidelines, audit, appropriate usage, inappropriate usage.

Abstract

Background: Demand of fresh frozen plasma (FFP) has increased substantially over the past few years due to increased therapeutic use in surgeries and liver diseases. Rational use of this blood component is essential in view of the perennial shortage and safety issues surrounding this precious resource. But this blood component is more often misused due to misconceptions regarding its hemostatic effectiveness and inadequate knowledge of the conditions in which its use is inappropriate.

Objectives: To carry out an audit of appropriateness of FFP usage in the Blood Bank of Rajindra Hospital, Patiala.

Methods: An audit of FFP usage was carried out at blood bank, Rajindra Hospital, Patiala over a period of six months. We evaluated all the FFP transfusion request forms to assess the rational usage of FFP and classified them as appropriate and inappropriate.

Results and conclusions: A total of 712 units were transfused to 189 patients during this period. Out of this, 301(42.23%) units were inappropriately used. Inappropriate requests were more from surgical specialties and the most common inappropriate indication for FFP use was burns. Continual medical education of the clinical consultants may help to reduce the inappropriate usage.

Introduction

Component therapy has had a profound impact on the practice of transfusion medicine. The extraction of various constituents, especially plasma, from whole blood has led to increased efficacy and economic utilization of the blood supply.

Fresh Frozen Plasma (FFP) is the fluid portion of one unit of human blood that has been centrifuged, separated, and frozen solid at -30°C or below within six hours of collection[1]. About 30% of solute component of plasma is made up of proteins; colloids, crystalloids and clotting factors. Plasma may be transfused up to 12 hours after thawing if stored at 2-4°C but contains slightly decreased levels of factor V (66+/-9%) and decreased factor VIII levels (41.8%).

The use of FFP has significantly increased in the past two decades and it is the most common inappropriately used product despite the existence of guidelines. The British Committee for Standards in Haematology (BCSH) 1992[2] and the College of Americans Pathologists (CAP) 1994[3] published guidelines to highlight these issues and minimize misuse.

FFP is mainly used in conditions with multiple coagulation factor deficiencies. Important limitations need to be borne in mind when prescribing FFP; half life of some coagulation factors is short, therefore FFP should be given close to the time of invasive procedure if correction of markedly prolonged prothrombin time (PT) or activated partial thromboplastin time (APTT) is required before surgery.

For specific factor or fibrinogen deficiency, the volume of FFP required for adequate replacement far exceeds that of specific factor concentrate or cryoprecipitate respectively, and should not be the preferred choice in these situations.

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FFP contains antibodies against ABO antigens and is capable of causing antibody-induced complications like hemolytic reactions, transfusion related acute lung injury (TRALI), allergic reactions and fluid overload[5]. It is also capable of transmitting viruses like human immunodeficiency virus, hepatitis B virus, hepatitis C virus and parvo virus although transmission of agents like herpes virus, malaria and cytomegalovirus has not been reported[4,6]. Hence the use of FFP is not without potential danger[5,6].

The current concerns for excessive use of FFP relate to preservation of resources, safety of the recipient and cost containment. The appropriate use of FFP requires an understanding of the properties of FFP and its inadequacies, as well as an appreciation of the complications of FFP usage. Many studies from around the world still report a high frequency of inappropriate usage[7-12].

Hence the decision to conduct an audit on the use of FFP in our hospital with the specific aims of assessing our pattern of usage and the rate of misuse. This will subsequently help us to recognize and solve problem sconcerning inappropriate use of FFP.

Material and methods

An audit of FFP usage was carried out at Department of Transfusion Medicine, Government Medical College and Rajindra Hospital, Patiala between Jan 2011 to June 2011 over a period of six months and details were obtained from the blood bank records. Data recorded include department requesting for FFP, reason for FFP request, date of transfusion, number of units transfused.

We evaluated all FFP transfusions, classifying them as appropriate or inappropriate according to the guidelines published by BCSH 1992 (Table I).

Results

A total of 778 units of FFP were issued during this period, out of which 66 units (8.4%) were returned unused and the remaining 712 units (91.5%) were actually transfused to 189 patients. The mean patient age was 37 yrs. with M: F ratio of 1.7:1. Only 411 (57.7%) of these transfused units were deemed appropriate based on the BCSH criteria. This meant that 301 (42.2%) units of FFP may have been in appropriately transfused. On an average each patient was given 3.7 units of FFP.

Table 1. Criteria for the use of fresh frozen plasma. Adapted from Transfusion Medicine Guidelines of the British Committee for Standards in Haematology 1992

Definite indications for the the use of FFP	**Conditional uses of FFP in the Presence of bleeding and disturbed coagulation**	**No justification for the use of FFP**
• Replacement of single coagulation factor deficiency where specific factor concentrate is not available	• Massive transfusion	• Hypovolaemia
• Immediate reversal of warfarin effect	• Liver disease	• Plasma exchange
• Acute disseminated Intravascular Coagulation	• Cardiopulmonary bypass surgery	• Nutritional support
• Thrombotic thrombocytopenic purpura	• Massive transfusion	• Treatment of immuno-deficiency states
• Replacement of single coagulation factor deficiency where specific factor concentrate is not available		• Hypovolaemia

Analysis of appropriateness of FFP transfusion for various indicationsare given in Table 2. Chronic liver disorders with bleeding, Guillain-Barrésyndrome (GBS) for plasmapheresis and disseminated intravascular coagulopathy (DIC) were the most common appropriate indications for FFP infusions. Of the patients with appropriate indications (n=87) for FFP, 74 (84%) had deranged coagulation profile. However, in only 61 (70.1%) of these patients, the adequate dose of FFP was given. The most common inappropriate use of FFP was in patients with burns and accidental trauma (circulatory volume replacement).

FFP is used by both medical and surgical units with general medicine, general surgery and burn wards being the main users (Table 3). Medical specialties used FFP more judiciously than surgical as shown in Table 4. Two hundred and fifty six (256) out of 281 (91.1%) FFP units used by the surgical units were inappropriate which was statistically highly significant (p=0.0001).

Table 2. Evaluation of FFP usage. (GBS – *Guillain – Barré*syndrome, DIC – Disseminated Intravascular Coagulation)

Diagnostic Criteria	**Appropriateness No of FFP units (%)**	**Inappropriateness No of FFP units (%)**
Chronic liver disease with bleeding	138 (19.3%)	—
GBS for plasmapheresis	64 (8.9%)	—
DIC	81 (11.3%)	—
Chronic renal failure with deranged coagulation profile	68 (9.5%)	—
Burns	—	93 (13.06%)
Surgical (preoperative)	—	76 (10.6%)
Factor deficiency and haemophilia	40 (5.6%)	—
Trauma	—	83 (11.6%)
Hyponatraemia	—	12 (1.6%)
Post operative bleeding with abnormal coagulation profile	20 (2.8%)	—
Others	—	37 (5.1%)
Total	411 (57.7%)	301 (42.2%)

Table 3. Distribution of FFP requests according to different departments

Departments	**No of total requests (%)**
General Medicine	270 (37.9%)
General Surgery	129 (18.1%)
Burn Unit	93 (13.06%)
Accident and trauma	83 (11.6%)
Gynaecology and Obstetrics	76 (10.6%)
Paediatrics	24 (3.3%)
Others	37 (5.1%)
Total	712 (100%)

Discussion

FFP is a frequently prescribed blood product. The use of FFP in hospital practice continue to rise but unfortunately, this product is overused or inappropriately used[21]. A high rate of inappropriate use of this product has been reported around the world[7-12]. The clinicians prescribing such transfusions should be aware of the correct indications for its use. Unlike red cell transfusions, where the traditional threshold of 10 gm/dl has been found to be unnecessarily high in some settings like[24] surgery and intensive care by prospective randomized studies, such studies do not exist for FFP usage. Even the threshold of prothrombin time (PT) and activated partial thromboplastin time (APTT) prolongation of 1.5 times normal was based on retrospective studies[13].

Concerns regarding transfusion-transmitted infections and non-availability of blood components in developing nations, make it crucial to optimize FFP transfusions and reduce wastage.

Inappropriate use leads to wastage of limited sources, deprives more needy patients of FFP, increases health care costs, and increase the risk of viral transmission, transfusion related acute lung injury (TRALI) and volume overload. Our study shows that inappropriate use of FFP was greater in surgical than medical services (Table 3). Other research studies has shown similar results[14,15]. This can be due to misconceptions of the haemostatic effectiveness of FFP and their underestimation of magnitude of risk associated with unnecessary FFP transfusion.

Besides almostall of the 66 unused units (8.8%) returned back to the blood bank were from surgical units, indicating inappropriate assessment of requirements for surgical procedures leading to over-ordering of this component. Novis et al. reported FFP wastage of 6.8% in the USA[16].

There are some situations where FFP is clearly indicated; bleeding patients or patients with acute DIC, liver failure, thrombotic thrombocytopenic purpura and massive blood transfusion.

Using the guidelines set by BCSH[2], 42.2% of FFP units were used inappropriately in our study. The most inappropriate use of fresh frozen plasma based on international criteria was in the burns ward, where FFP was used as replacement of massive protein loss. This is done because synthetic albumin is either not available or too costly. In massive bleeding, it has been shown that there is no indication for FFP unless the blood loss is in excess of 5000 ml[25]. There is absolutely no justification for the administration of FFP as volume expander. This is the second inappropriate use of FFP. It is also used as a nutritional supplement. Safer alternatives, such as crystalloids, synthetic colloids or human albumin solutions are available for volume expansion. However, the high cost of human albumin discourages its use in hypoproteinemia in developing countries.

Table 4. Comparison of appropriate usage between medical and surgical specialties

Department Services	Total FFP infused (%)	Appropriate use (%)	Inappropriate use (%)
Medical	431 (60.5%)	386 (89.5%)	45 (10.4%)
Surgical	281 (39.4%)	25 (8.8%)	256 (91.1%)
Total (n=189pts)	712	411 (57.7%)	301 (42.2%)

χ^2 454 df 1 p value 0.0001 Highly significant

In our study, we also found that plasma was used inappropriately in conditions like coronary bypass surgeries, infections and some clinical situations where indications of plasma was either vague or could not be defined.

The routine use of FFP in coronary artery bypass (CABG) is not recommended. Routine prophylactic administration of FFP in uncomplicated elective CABG does not reduce the blood loss or transfusion requirement during or after surgery. Such patients may bleed postoperatively due to surgical causes like platelets dysfunction and residual effects of heparin[17]. FFP should be used only with proven coagulation abnormality.

FFP is not recommended as a means of transfusion of immunoglobulins during infections as commercially prepared immunoglobulins are available[5]. The use of such products minimize the risk of viral transmissions too. Our study showed that 8 units of plasma were used in three patients suffering from different infections.

In surgical services, the prophylactic transfusion of FFPs in patients with normal coagulation profile before or after procedures, was noted. In present study, 76 (10.6%) FFP units were used preoperatively with normal coagulation profile hence was inappropriate.

Chronic liver disease is one of the most common clinical indications for transfusion of FFP. The patients with liver diseases have several abnormalities that can lead to bleeding like coagulopathy, thrombocytopenia and surgical causes as esophageal varices. It is recommended that FFP should be given only when bleeding has taken place due to impaired coagulation or when surgery is anticipated in the patients with liver diseases[18,19]. In our study, 138 units (19.3%) were used for patients of chronic liver disease with bleeding and was appropriate.

Other common indications for FFP usage is disseminated intravascular coagulopathy (DIC). The use of FFP is appropriate in DIC where there is activation of the coagulation system with consumption of clotting factors leading to a generalized coagulopathy but according to CAP guidelines, FFP should be given only in the settings of bleeding in these patients. In present study, 81 (11.3%) FFP units were used for this condition and was classified as appropriate.

The high rate of inappropriate transfusions reflects poor understanding of the indications of use of FFP. It is reflected by the results, as only 21 (11.1%) of the total 189 requests had either PT/APTT values more than 1.5 times normal.

Many audits have been done on the usage of FFP. Reported prevalence of inappropriate use range from 20% to 73%[20-22]. Sixty four percent of the FFP prescriptions were found inappropriate by Pervaiz and Lubna in tertiary care hospital in 2009[23].

A similar audit was done at Venezuela General University in 1999 showing 47.8% appropriate transfusions using the same guidelines[11]. Using BCSH guidelines, 60.3% FFP prescriptions were found inappropriate by Kakkar et al in 2004[7] which however reduced to 26.6% after educational campaigns of clinicians. Similarly 34% of patients appeared to have received FFP for reasons not clearly within these guidelines in an audit by National blood service hospitals in United Kingdom[24]. Similar results were shown by Kaushal et al in 2011[25].

FFP misuse results in wastage and subjects recipients to unnecessary risk. Despite guidelines, inappropriate FFP use is a significant problem worldwide, in developed and developing countries. Our audit provides further evidence that this is an on-going problem. Our study showed lack of knowledge about the appropriate use of FFP amongst our clinicians especially surgeons.

We recommend that educational programs be arranged for doctors prescribing FFP regarding its' appropriate use to minimize inappropriate use. We also recommend that periodic auditing be done in the blood banks to keep a track of utilization of this precious blood product.

Authorship

Contribution: All authors have contributed equally. All authors approved the final version.

Conflict-of-interest disclosure: The authors declare no conflict of interest.

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Answers for CME: The clinical significance of the new parameters of the reticulocyte

1) c

2) d

3) e

Explanations

Q 1. Best answer is "c". i.e. Reticulocyte Production Index (RPI)

Hb of 6.5g/dl in a 3rd trimester female, not on haematenics, could be due to many reasons; e.g. haemorrhage, haemolysis, mixed deficiency, pregnancy induced aplasia.

RPI > 3 is seen in anaemia with adequate regeneration, e.g. haemorrhage / haemolysis.

RPI < 2 is indicative of anaemia with inadequate regeneration, e.g. aplasia / deficiency states.

Normal value is 1.

Q 2. Best answer is "d" i.e. immature reticulocyte fraction (IRF)

The IRF value is an early marker for evaluating the regeneration of erythropoisesis. Whereas the IRF percentage increases after only a few hours, the reticulocyte count increases after two to three days.

Together, the IRF value and the reticulocyte count have proven themselves as monitoring parameters for bone marrow and stem cell transplants. In successful transplants in 80% of cases the IRF value reaches its 5% mark earlier than the granulocytes their classic threshold of $0.5x10^9$ granulocytes/L.

Q 3. Best answer is "e", i.e. Reticulocyte Hb content (CHr).

This will show if there is iron deficient erythropoiesis. Thus additional iron supplements can be given early to improve the functional iron deficiency which may not be evident with iron studies alone.