The field of Clinical Haematology is a rapidly evolving specialty. The clinical entities encountered in everyday practice have basically not changed, but our understanding of them and the biological and molecular processes that drive them are undoubtedly much better understood. Our classification of these diseases has been continually refined over the decades, with the WHO classification of tumours of haematopoietic and lymphoid tissues now being firmly established as our current working reference manual [1]. The introduction of the WHO classification of haematological malignancy provides a structure for the development of integrated haematopathology laboratories, with its emphasis on definition of disease entities based on clinical, morphological, phenotypical and molecular features.

The real fascination of diagnostic clinical haematology lies in the diversity of the entities that we encounter. The whole spectrum of abnormal haematological parameters triggered by a variety of reactive and neoplastic processes are studied in the diagnostic laboratory. Flow cytometers are no longer a technical novelty confined to a few highly specialized diagnostic institutions. The flow cytometer methodology is well known, cytometers are readily available and whilst its applications are constantly diversifying its contribution to routine diagnosis is widely appreciated. This technology provides objective, rapid, sensitive and accurate measurement of a broad range of cell characteristics.

Accurate diagnosis is clearly of utmost importance in making decisions on patient management. The cognitive pathways to explain the processes of achieving a diagnosis, have in our opinion, however, been somewhat neglected in the haematological literature. There are many excellent texts which describe the clinical, haematological, immunophenotypic, cytogenetic and molecular characteristics of a wide variety of neoplastic disorders. These texts are ideal reference manuals to study once a diagnosis has been achieved. The missing element, however, is a systematic approach to immunophenotypic diagnosis, taking into account all available clinical and laboratory data. In laboratory diagnostics, we are often left with the chore of finding the missing piece to the jigsaw puzzle. This should not be through a process of aimless searching but should be achieved through a logical carefully considered approach. What this publication aims to achieve is to generate a readable text to clearly explain the principles by which these diagnoses can be achieved, how immunophenotypic data can be analysed in clinical context and how meaningful conclusions can be drawn. We focus on clinical flow cytometry analysis of normal and malignant cells, not just in blood and bone marrow, but also in extramedullary sites such as effusions and cerebrospinal fluid. Each of these specimens needs to be handled, analysed and interpreted in a specific way. Of utmost importance is the assessment of features of the clinical history, physical examination, biochemical, immunological and radiological findings of a clinical case in relation to the current haematological parameters. We make no apology for this, as we believe this is of ultimate importance and this principle will be encountered repeatedly throughout this text. This approach, as recommended by the National Institute of Clinical Excellence [2], suggests that the diagnosis of leukaemia and lymphoma should take place in a specialist laboratory and in most cases this should be organized on a regional basis with full access to, and interaction with, diagnostic histopathology, cytogenetic and molecular laboratories with expertise in this field.

Flow cytometry analysis, used to determine the nature, origin and behaviour of cells if carefully directed can be a magnificent diagnostic tool and is exquisite in
categorizing cell populations present at low levels through the analysis of cell size, complexity and granularity in relation to the expression of surface, cytoplasmic and nuclear antigens. Many thousands of cells can be so categorized over a short timeframe. It cannot, however, be applied in isolation and flow cytometry, if poorly directed, can lead to erroneous conclusions. For example, identification of the diseased cell population is usually easy in a bone marrow aspirate in a patient presenting with pancytopenia due to acute leukaemia. The disease cells may not be apparent in peripheral blood but are abundant in bone marrow and are identified and categorized using a carefully chosen myeloid panel. In contrast, the disease cells in hairy cell leukaemia may form a minority population in peripheral blood in a second patient with pancytopenia. The bone marrow aspirate is often dry and uninformative. Immunophenotyping using a mature lymphoid panel will provide a diagnosis. Unless the material is carefully scrutinized and the clinical presentation taken into account, then immunophenotyping can be misdirected and the wrong conclusions might be drawn.

We aim to explain from those most basic steps, how to approach clinical flow cytometry analysis of a variety of clinical specimens, to highlight the strengths and pitfalls, and how to safely embark on this fascinating diagnostic process in a variety of clinical circumstances. We aim to cover reactive phenomena, which in our opinion have not been well covered in the world literature. We aim to illustrate to the student that there is clear logic to explain the immunophenotype of any clonal condition and that they should understand this basis and not attempt to remember an immunophenotype as a random set of CD numbers. This latter approach is bound to fail. The former approach will establish a firm understanding and foundation on which to build and will assist in suggesting additional immunophenotypic studies or ancillary investigations in situations where the diagnosis is not immediately apparent. In addition to the diagnosis of leukaemia and lymphoma we look at flow cytometric applications to response assessment and quantification of minimal residual disease. Finally, we also look at FCM analysis with respect to the diagnosis of red cell, granulocyte and platelet disorders.

This text is written for trainee and practising haematologists, haematopathologists and biomedical scientists with a specific interest. It should assist in preparation for FRCPATH UK in Haematology and is intended as a working manual for diagnostic laboratories throughout the world. The chapters are illustrated with morphology images, scatter plots, cytogenetic and molecular data from real clinical cases – often a carefully chosen image will illustrate a principle much more succinctly than a thousand written words.

The authors have done their utmost to ensure the accuracy of data presented in this text. In fact, one of the driving forces in undertaking this exercise was to provide a practical handbook that would assist in the safe and accurate use of flow cytometry as a diagnostic tool. The content is extensively researched and referenced but also relates to personal experience of the authors who interpret flow cytometry on a daily basis in a regional reference centre. We sincerely hope that our readers will find it of interest and of practical value when applied to haematology diagnosis. We cannot, however, accept any responsibility for any error or misinterpretation which might, in any way, arise from its use.

References