PROGRESS AND CHALLENGES IN AFRICA AT THE TIME OF MOLECULAR HAEMATOLOGY

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ABSTRACT

The study of blood diseases has been traditionally at the border between clinical medicine and laboratory science. Many of us have been attracted to haematology by this combination: by the very fact that from examining a patient and looking at the blood picture it is often possible to make a diagnosis. As for understanding the nature of any disease, there are several layers: but since the middle of the last century, when the phrase 'molecular disease' was coined to aptly describe the basis of sickle cell anaemia,[1] it became clear that in understanding the causes and the pathogenetic mechanisms of any disease the ultimate layer must be molecular.

From the middle of the last century African pioneers of haematology have been prominent: without detracting from others, let me mention Alex Boyo in Nigeria,[2] Felix Konotey-Ahulu in Ghana,[3] Edward Kasili in Kenya,[4] Aloysius Nhonoli in Tanzania,[5] Charles Olweny in Uganda[6]: as I have been fortunate to know each one of them in person. As clinical and epidemiological studies were being conducted, the pattern of blood disorders in sub-Saharan Africa (SSA) gradually emerged, and it had several important features. First, as could be expected, an African patient with megaloblastic anaemia, or one with thrombocytopenic purpura, or one with Hodgkin disease, or one with any other specific blood disease was not much different from a patient with the same disease in any other part of the world. Second, in contrast to clinical similarity, the prevalence of many blood conditions was very different: first and foremost, anaemia was and still is rampant,[7] due to a variety of infectious and nutritional causes, and it is of great clinical importance particularly in children and in pregnancy.[8] Third, whereas in USA and in Europe haemoglobinopathies are officially 'rare diseases', in many countries in SSA the prevalence of sickle cell disease (SCD) at birth is from 1 to 2%: with millions of people affected, it is a major public health problem. Fourth, a unique haematologic malignancy, Burkitt's lymphoma, was discovered in Africa.[9] Last, in SSA, and especially in West Africa, endemic malaria, in addition to being a major cause of mortality in children, is always in the background: during my first couple of years at UCH in Ibadan, when I regarded myself as being still very much in training, I learnt that when a patient had a fever the question was not whether he or she had malaria: rather, whether it was *just* malaria or also something else on top.

Keywords - Traditional haematology, molecular haematology, haemoglobinopathy, haematological malignancy, Nigeria, Africa.

Traditional haematology and molecular haematology

From the clinical point of view, it makes no difference whether a patient is investigated by a molecular approach or by traditional methodologies, provided a diagnosis can be reliably established. For instance, it is clearly not necessary to demonstrate by DNA sequencing that a patient has a *HBB*^{E6V} mutation in double dose to diagnose sickle cell anaemia: haemoglobin electrophoresis can be just as definitive (see below), and some point of care (POC) tests are also getting there. On the other hand, in an infant with persistently low granulocyte counts and recurrent bacterial infection one can suspect severe congenital neutropenia (SNC): but a firm diagnosis of SNC can be made only by finding a mutation of *ELANE*[10] or other relevant gene.

The change from traditional medicine to molecular medicine is sometimes depicted as though it was a sudden revolution; in reality all such transitions are gradual. In science and in medicine progress generally takes place step by step; some steps are taller than others, but sometimes even a small step may be important. Old tools may be replaced by new ones, but sometimes it is wise to regard a new tool as an addition rather than a replacement. Here we will try to outline a few individual examples, relevant to SSA, of how understanding blood disorders at the molecular level has impacted haematology; and what challenges we are still facing.

Haemoglobinopathies

Diagnosis

Since SCD was the trailblazer, it is natural that it should be our first example. For the diagnosis of the major haemoglobinopathies, haemoglobin electrophoresis is still pretty good: it displays a single Hb S band in homozygous sickle cell anaemia, it displays two well resolved bands in haemoglobin S/C disease, and it differentiates unambiguously sickle cell trait from sickle cell disease (see Fig. 1). S/β^+ thalassaemia is given away by the co-existence with the Hb S band of a minor Hb A band, usually associated with an obvious increase in Hb F. S/β^0 thalassaemia is trickier, because it requires demonstrating an increase in Hb A_2: in most labs quantitation of haemoglobins is carried out nowadays by high performance liquid chromatography (HPLC), but unfortunately by this technique Hb A_2 is systematically over-estimated in the presence of Hb S [11]. Therefore, in the end only identifying a β along with the *HBB*^{E6V} mutation, provides a definitive diagnosis. As for the coexistence of ✔-thalassaemia, whether in single dose or in double dose, this can be suspected from the red cell indices (low MCV and low MCH with normal MCHC): but again, it needs molecular analysis for definitive confirmation. Recently this has proven feasible and relatively inexpensive in Tanzania by the use of nanopore technology.[12]

Epidemiology and evolution

Haemoglobin S has a worldwide distribution (Fig. 2); it has been a model not just in molecular medicine, but also in our understanding of human evolution, as it is the best characterized instance of a genetic polymorphism in which the severe phenotype of homozygotes is balanced by protection against malaria in heterozygotes.[13] The main mechanism of protection, first outlined a long time ago in Nigeria,[14] is the early removal of *P falciparum*-infected red cells by phagocytosis. Although the *Plasmodium*-erythrocyte interactions are complex, no evidence for any alternative mechanism has ever materialized: indeed, Ayi et al[15] confirmed by *in vitro* culture experiments that *P falciparum*-parasitized AS red cells, compared to *P falciparum*-parasitized AA red cells, are removed by macrophage at a much earlier stage in the parasite cycle: thus confirming the mechanism originally proposed.[16] Malaria

selection for AS heterozygotes is still on-going:[17] it can be so powerful that, wherever the *HBB*^{E6V} mutation occurs, in the presence of endemic malaria its frequency will increase. Since this mutation has been seen in different haplotype contexts within Africa, it was suggested that at least three independent mutational events have occurred.[18] However, a study based on full-sequence haplotypes has shown that the data are more compatible with a single origin of the Hb S gene in Africa (Fig. 3), that may have taken place 7,300 years ago (corresponding to 259 generations), during the 'Holocene Wet Phase', when the areas in Africa that are now deserts were still humid.[19]

There is a vast literature on SCD (n > 33,000 in *PubMed*), and I will certainly not remotely attempt to summarize it here. In a recent comprehensive review[20] Authors from Africa have been prominent. SCD is officially a 'rare disease' in Europe and in USA; it is commonplace to say that it is not at all rare in Africa but, if we try and visualize the size of the problem, we find that this is still an under-statement. In Nigeria (population 223 million) approximately 1.5% of babies are born with SCD, and therefore if their survival were the same as that of the general population, there would be in the country some 3.3 million SCD patients: they are less because of earlier mortality (by comparison to another major public health problem, the number of HIV patients in Nigeria is estimated to be 1.9 million[21]).

Pathophysiology

SCD has a number of unique characteristics. First, there is a paradox: although the commonest form of SCD is called sickle cell anaemia, in the majority of patients the main clinical problem is not anaemia: rather, the occurrence and recurrence of the painful episodes, often true attacks, that we call vaso-occlusive crises (VOC). Second, SCD is a paradigm of a haemolytic anaemia due to an intrinsic red cell abnormality: it has been recognized for a long time that the mechanism of red cell destruction is both extravascular and intravascular,[22] and the importance of the latter has been highlighted more recently.[23] Third, SCD entails an associated chronic inflammatory state,[24] of which we find evidence even in an ordinary blood count, whereby neutrophils and platelets are very often elevated.

There can be little doubt that all the above features are ultimately a consequence of sickling, the unique phenomenon that in turn results from polymerization of deoxy-haemoglobin S. Whereas investigation of the mechanisms of these individual features has been on-going for decades,[25] in recent years there has been also a well-directed interest in making use of our understanding of pathophysiology for the purpose of identifying possible targets for pharmacologic therapy (see Fig. 4).[26]

SCD is unquestionably a monogenic disease: yet, not surprisingly, its clinical expression is influenced by variation in genes, other than *HBB*, that are referred to as having epistatic effects, or as being modifier genes. This important matter has been investigated in several genome-wide-association studies (GWAS), including one on nearly 2000 Tanzanian patients with SCD.[27] A recent comprehensive systematic meta-analysis[28] has confirmed, as one might have expected, that the most reproducible evidence for genetic modifiers that affect the severity of SCD regards (i) variants in genes (*BCL11A, HBS1L-MYB, HBG2*) regulating fetal haemoglobin (Hb F: a fourth, X-linked gene affecting Hb F has been also reported through a gender-specific genome-wide association study[29]); and (ii) ✔-thalassemia. Interestingly, no comparable level of evidence has emerged for other genes; however, pathway analyses have highlighted the importance of cellular adhesion,

inflammation, oxidative and toxic stress, and blood vessel regulation. Again, these data may suggest potential therapeutic targets.

Therapy

Given the daunting figures regarding the prevalence and incidence of SCD in Nigeria, we are confronted with a dilemma that is both ethical and practical. On one hand, the dictate of the Hippocratic oath is that we must do the best for each individual patient, regardless of who and where; on the other hand, resources are from limited to minimal. As a general principle in medicine, a cure is preferable to a measure that merely ameliorates a disease: we have known for decades[30] that SCD can be cured by bone marrow transplantation (BMT), and this is practiced in Nigeria.[31] However, even in high income countries (HIC) only a small fraction of SCD patients receive BMT: in part because few patients have an HLA-identical sib – still today the preferred donor; but also for a variety of other reasons.[19,32] Today SCD can be also cured by gene therapy (see Fig. 5A):[33] a most elating development, not only because it is a logical approach to the correction of a monogenic disease, made possible by contemporary technology; but also because, by using the patient's own hematopoietic stem cells, it bypasses any immunologic complications, such as graft rejection and graft versus host disease. This *ex-vivo* gene therapy (GT) is much more recent than BMT; already an alternative CRISPR-based approach (still *ex-vivo:* see Fig. 5B) has been introduced,[34] and has recently obtained FDA approval in the US. To date numbers are small and follow-up rather short;[35] and the fact that two patients unfortunately developed haematological malignancy deserves close scrutiny,[36] keeping in mind that leukemia can develop in a patient with SCD quite regardless of gene therapy procedures, as exemplified by a well-documented case in Nigeria.[37] The infrastructure requirements for BMT and GT are similar, and they are already met in a few sites in SSA. However, on top of the infrastructure both procedures are labour-intensive and highly expensive; and given the numbers quoted above, even regardless of costs, it is unconceivable that either can be offered in the foreseeable future to more than a minute fraction of patients with SCD. Partly for this reason, there is a strong drive to the development of *in vivo* GT for SCD:[38] this is at the moment a research endeavour in which strides are being made,[39] but still at an early experimental pre-clinical stage.

With respect to medicines that do not cure, but can ameliorate SCD, there has been progress recently. P-selectin, a trans-membrane protein, promotes adherence of leukocytes to activated platelets and endothelium:[40] therefore, it may mediate inflammation. P-selectin, expressed on the surface of the endothelium, also mediates abnormal rolling and static adhesion of sickle erythrocytes to the vessel surface,[41] and therefore it may play a role in triggering VOC. This was the basis for the development of *crizanlizumab*, a monoclonal antibody that binds to P-selectin, and that has been found to reduce significantly the frequency of VOCs in patients with SCD.[42] *Voxelotor* (formerly GBT440) was synthesized by organic chemistry as part of a deliberate search for a small molecule that would stabilize the oxy-form (the so called relaxed or R-state) of haemoglobin:[43] thus, it shifts to the left the Hb-O_2 dissociation curve. Since polymerization causing sickling is a prerogative of deoxy-Hb S, by decreasing this form of Hb voxelotor inhibits polymerization and therefore sickling. In a clinical trial voxelotor has been found to increase hemoglobin levels and decrease hemolysis.[44] A physiological intra-erythrocytic regulator of the Hb-O_2 dissociation curve is bis-phosphoglycerate (BPG). *Mitapivat*, a small molecule that activates pyruvate kinase

(PK), causes a decrease in BPG, thus also shifting the oxygen dissociation curve to the left. Mitapivat has been approved for the treatment of haemolytic anaemia caused by PK deficiency, and in a recent pilot trial in 9 patients with SCD it has produced a significant increase in Hb levels.[45] These three medicines have had the considerable benefits provided by the *Orphan Drug* legislation, without which probably they would not exist. However, at the moment the prices are as follows: crizanlizumab about $241 per day, voxelotor about $342 per day, mitapivat about $915 per day. It is too early to know how helpful these medicines will be in real-life practice: and, unless the prices are reduced by at least two orders of magnitude, we shall never know, because they will remain irrelevant to almost all SCD patients in Nigeria.

Regardless of the above, a time-honoured ameliorative medicine does exist: hydroxyurea (HU), that has been proven effective for some three decades[46] and, as expected, is just as effective in Africa[47] (see Fig. 6); unexpectedly, it also reduces malaria.[48] Nevertheless, it is estimated that less than 20% of patients with SCD in this continent receive HU regularly: there may be several reasons for this serious anomaly,[49] including sometimes reluctance by patients. However, when galenic HU capsules were produced in Tanzania and provided free of charge, not a single patient declined,[50] supporting the notion that by far the major barrier is economic; and a Nigerian pharmaceutical firm has made HU available at low price.[20] In my view to provide HU to all SCD patients is a **must,** and the most urgent priority in the management of SCD in Africa:[51] some suggestions about how to implement this task have been formulated (see Table 1).[52]

Haematologic malignancy

Any haematologist from outside Africa who reads this heading is likely to think immediately of Burkitt's lymphoma; but any haematologist from Africa knows that even in this continent, other types of lymphoma are more common. In a recent report on adult hematologic malignancies in Nigeria,[53] leukaemias accounted for 48%, lymphomas for 36%, and multiple myeloma (MM) for 7%. Patients with leukaemia have been investigated systematically in some African centres for decades: see Fig. 7.[54]

It is possible that in the past many patients with leukaemia, especially acute leukaemia, may have died without a diagnosis. Nowadays there are trained haematologists in all African countries: in Nigeria I believe about 300, and probably at least as many in training. Even though probably still less than recommended by professional bodies in Europe or USA, this number is more than respectable: therefore, referral of patients with suspected acute leukaemia and definitive diagnosis must have increased considerably. On the other hand, when it comes to treatment, I am very aware of and sympathetic with respect to the limitations that still exist.

In a previous article[55] it was suggested that each hospital sets its own policies with respect to hematologic malignancies, by first classifying them into three categories: (A) conditions that can be dealt with adequately; (B) conditions for which one can offer treatment that may not be optimal, but is nevertheless of proven efficacy; (C) conditions for which appropriate treatment cannot be offered, whereby the patient, if possible, should be referred elsewhere. Major factors that will influence this categorization are (i) availability of drugs, (ii) supporting services – particularly microbiology and availability of platelet concentrates – and (iii) availability of BMT. Clearly these factors ultimately depend on the hospital's finances; at the same time,

haematologists in each hospital, especially in each teaching hospital – ought to be pro-active in demanding and fostering the development of these facilities. The above categorization, far from being definitive, must be revised all the time: the aim must be to gradually move more and more conditions from (C) to (B), and from (B) to (A).

We can consider just two examples. The management of chronic myeloid leukaemia (CML) has been transformed since the introduction of the tyrosine kinase inhibitors (TKI), of which imatinib has been the trailblazer. For some twenty years the *Glivec International Patient Assistance Program* (GIPAP), consisting of a partnership between *Novartis* and the *Max Foundation*, has provided imatinib free of charge for patients in several LMICs with a diagnosis of CML confirmed by cytogenetic or molecular analysis: as a result, in hospitals in Africa that have been able to thus diagnose CML, this condition has progressed from class B to class A, at least with respect to up-front treatment. This transition has greatly benefited patients, and it has also contributed to novel findings important for our understanding of CML. Owojuyigbe et al[56] have measured quantitatively the *BCR-ABL* gene transcripts, and they have determined that in Nigerian patients the co-expression of the e13a2 and e14a2 transcript variants is higher than that reported thus far in any other populations. In Tanzania Nasser et al[57] have found that upon treatment with imatinib only 23% of patients with CML achieved optimal molecular response: this was probably due in large part to late presentation with very bulky disease (see Fig. 8). I remember two patients who had a total white cell count of 830,000 and 1,123,000 respectively: top CML expert in Europe or USA may never have come across this nowadays, although such counts were seen in the past.[58] Indeed, this is not limited to CML: within haematologic malignancies in general patients not previously treated are seen in Africa, on average, at a more advanced stage than in Europe. The reasons for delay in treatment of lymphoma have been analysed in East Africa,[59] and this must be a stimulus to prioritize earlier diagnosis; at the same time, we should not bypass the unique opportunity to investigate by contemporary methodologies the features of advanced disease, including their pathology, in Africa, as it is rarely done elsewhere.

Another example is multiple myeloma (MM): a condition in which the multi-step pathogenesis that underlies almost every form of malignancy is tangibly illustrated by the fact that it is always preceded by a monoclonal gammopathy of (so-called) uncertain significance, or MGUS. For a long time it has been known that in the USA the incidence of MM is about twice as high in African Americans than in the general population [see US Surveillance, Epidemiology, and End Results (SEER) Program, https://seer.cancer.gov/statfacts/html/mulmy.html]. Nevertheless, in a recent worldwide epidemiological survey[60] the incidence of MM in most African countries was low. This finding is almost certainly due to under-reporting, since in a valuable study in Ghana the frequency of MGUS in men was in fact about twice that recorded in white men elsewhere.[61] It is possible that genetic factors may account for a higher frequency of MM in African people: a deliberate genome-wide association study is currently being planned and it is hoped it will recruit sufficient numbers of patients (Anastasios Karadimitris and Julie Makani, personal communication). As for outcomes, the estimate by SEER of the overall 5-year survival for MM patients in the US is 59.8%; whereas In a retrospective study from Nigeria the 5 year-survival was 8%.[62] From this study it emerged that more than one-half of MM patients had received only melphalan and prednisone; since these two drugs are generally available, whereas

bortezomib and lenalidomide are less widely available,[60] in most hospitals within SSA MM is in the above category B: thus, there is room for improvement. Unfortunately, MM is still an incurable disease – not only in Nigeria; and in a large proportion of patients autologous BMT (aBMT) is part of optimal treatment: this procedure has been practiced extensively for many years in RSA, where it has been carried out largely on an out-patient basis and it has been proven effective (>60% 5 year-survival), and also cost-effective.[63] aBMT is currently being introduced, within SSA, in several hospitals, that will be able to promote MM to category A (see Table 1).

As for paediatric haematology, in Africa like elsewhere precursor B-cell acute lymphoblastic leukaemia (ALL) is probably the commonest malignancy in children; although in a report by the *French African Pediatric Oncology Group* Burkitt lymphoma was ahead.[64] A remarkable feature in the management of childhood ALL is that the backbone agents – such as vincristine, prednisone, l-asparaginase, methotrexate – have remained the same for decades:[65] although several improvements have been introduced since, both in assessing response to induction and in the consolidation and maintenance phases of the treatment, lasting a total of some 30 months. In a recent paper that has deliberately aimed to compare modalities of management of childhood ALL in different countries,[66] the current long-term survival in Tanzania (P Scanlan et al.) is at least 50%. This has been achieved thanks to rigorous and meticulous adherence to treatment protocols, which is imperative; and also, through close interaction with a charity organization that has greatly helped to provide treatment free of charge; without which it would have been impossible to overcome the divide between those who can and those – the majority – who cannot afford to pay.

Other blood diseases

Haemophilia A is the most prevalent serious genetic disorder of haemostasis: it has a worldwide distribution and it has been studied in Nigeria for over fifty years.[67] The formation of factor VIII inhibitors, *i.e.* anti-factor VIII antibodies, is the most severe complication of replacement therapy in patients with haemophilia A. Why some but not all haemophilia patients develop these antibodies has been largely clarified, once again, by molecular studies: patients with *F8* mutations that result in an absent or truncated factor VIII protein (mainly large deletions and intron 22 inversions) are associated with a higher risk of inhibitor formation.[68] A most significant advance in managing patients with inhibitors has been the introduction of emicizumab: this bi-specific antibody is able to bring about the coupling of factor X with activated factor IX, thus surrogating artificially the function of factor VIII. Emicizumab is a recent artful product of advanced biotechnology: it could not have been developed but thanks to the previous one century of research that has elucidated the coagulation cascade. In trials conducted in many haemophilia centres, including one in Johannesburg, emicizumab has proven highly effective in treating and preventing bleeding in haemophilia A patients, whether they have an inhibitor[69] or not[70]. In Africa the management of haemophilia has been always problematic because of the high prices of medicinal coagulation factors; however, the *World Federation of Hemophilia* (WFH) has expanded since 2015 its *Hemophilia Aid Programme* (HAP) and has made these products available in most countries in Africa, including Nigeria.[71] In 2020 two patients in Zambia were the first in Africa to receive emicizumab, and the pharmaceutical company Roche has promised the Haemophilia Foundation of Zambia to provide this drug to 1000 more patients over

the next 5 years (https://wfh.org/article/zambia-first-country-to-receive-donated-emicizumab/); subsequently a study in Ivory Coast reported on 33 young patients with haemophilia A who had a good response to emicizumab.[72]

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired disease, much more rare than any of the above, characterized by the classic triad of (i) haemolytic anaemia, (ii) high risk of thrombosis in atypical sites, and (iii) an element of bone marrow failure. It was work in Nigeria that provided the first evidence that PNH is a clonal disorder,[73] and it can be regarded still today as a paradigm of a non-malignant clonal disorder. The clone arises through a somatic mutation in a haematopoietic stem cell of the X-linked gene *PIGA* [74], required for the biosynthesis of the glycosyl-phosphatidyl-inositol (GPI) molecule that anchors many proteins to the cell membrane. However, the *PIGA* mutation on its own does not cause PNH: the disease will develop only if the *PIGA* mutant clone expands, often to the extent of taking over most of the patient's haematopoiesis.[75] Clonal expansion is related to the bone marrow failure component of PNH: the GPI-deficient *PIGA* mutant clone expands[76] as a result of the GPI-normal stem cells being selectively damaged by a T-cell mediated auto-immune attack, as in aplastic anaemia (AA) [77]. The expansion of the PNH clone brings about essentially a self-cure of PNH: but because the complement regulatory surface proteins CD55 and CD59 are GPI-linked, this cure comes with the payload of exquisite susceptibility of red cells to activate complement: hence the haemolytic anaemia. Both AA and PNH are serious conditions that are increasingly diagnosed in Africa, where they are no more rare than elsewhere:[78] see Table 2. The treatment of AA is demanding, as it requires anti-lymphocyte globulin or allogeneic BMT. The management of PNH has been mainly supportive until the introduction of eculizumab, an antibody that targets complement component C5:[79] this has transformed the clinical features of PNH, by curbing intravascular haemolysis and reducing markedly the risk of thrombosis. Unfortunately, the price of eculizumab, and of other more recent anti-complement drugs[80,81] is astronomic, and therefore these medicines are not yet available in Africa and in other parts of the world. In my view we should operate pro-actively to redress this injustice (see Table 1).

Concluding remarks

From this brief overview of only a few examples of major hematologic conditions, it is clear that today the molecular approach is pervasive: indeed, one might say that contemporary medicine cannot be *but* molecular. DNA analysis can tell us the precise basis of inherited diseases, and it can tell us the basis of neoplastic and non-neoplastic diseases arising from somatic mutations. Other 'omics' technologies can give us comprehensive information on epigenetic changes[82] or on gene expression, even at the level of single cells;[83] and in many cases flow cytometry has become part of the diagnostic work-up.

Partly as a result of these advances, and especially thanks to the training of doctors in the haematology specialty, the situation of patients with blood diseases in most African countries has changed substantially, when compared to what it was when I worked at UCH, Ibadan (1964-1974). The most significant improvement has been, in my view, especially with respect to diagnosis, and also with respect to treatment; however, optimal management is still frequently compromised by significant barriers; and in some cases, this can make management of patients even more frustrating than when we did not have a diagnosis. The word *barriers* have become recurrent in the literature, certainly not only In Africa; but when barriers

are analysed, they often boil down to the less elegant word money. When I was trained, the attitude of most of my teachers was that money was an issue for the administrators, not for the doctors; but over two generations, since I trained, I have become convinced that doctors ought to be more pro-active in this respect. Clearly in every country how much to invest in petroleum, green energy, automobiles, military equipment, agriculture, health is the subject of political choices: but this does not exempt doctors from making efforts to influence those choices.

Coming back to science, we should not presume that just because we know the molecular basis of a particular disease, we have understood all of its aspects: for instance, we still don't know when a patient with SCD will develop a pain crisis; we still don't know why one patient has transformed from MGUS to MM within 2 years, and another patient, after 20 years, has still not transformed: we can only say that this transformation (and perhaps also the onset of pain crisis in SCD) behaves like a stochastic phenomenon. We do know that in SCD a higher number of F cells is associated with generally less severe disease, but several aspects of pathophysiology still need investigation: for instance, the relative roles, in individual patients, of intravascular *versus* extravascular haemolysis. At the practical level, as automated equipment is becoming more popular, it would seem important to investigate objectively what is the most cost-effective way to provide platelets and other blood products when giving a course of anti-lymphocyte globulin to patients with aplastic anaemia.

Thus, there is ample room for young haematologists anywhere in the world to become engaged in research: this is part of the mission of the *Nigerian Society of Haematology and Blood Transfusion*. To reconcile, in a busy haematology unit, the demands of the clinic and of the laboratory with dedication to research work is not easy in any country: but this is the challenge for the future, as it has been in the past. Research is about discovering something that was not yet known, and I am confident haematologists in Nigeria will continue to meet that challenge.

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Table 1. Attempts to correct for unmet needs in Africa.

Condition	*Drug/medical procedure*	*Proposal*	*Notes*
Sickle cell disease (SCD)	Hydroxyurea	Adding SCD to the triad of conditions (HIV, tuberculosis, malaria) for which cost of treatment in Africa is born by the Global Fund	SCD, like the other three conditions, is a **major public health problem in Africa**
Multiple myeloma, acute leukemia, SCD	Autologous and allogeneic bone marrow transplantation (BMT)	For every BMT procedure in Europe/US, 0.1% of the expense is to be deposited into a fund to support BMT in accredited centres in Africa	**BMT solidarity programme**
Paroxysmal nocturnal haemoglobinuria and other rare diseases	Eculizumab; other long-term medicines	For every 10 patients treated with a super-expensive drug reimbursed by NHS/insurance, the manufacturer offers the drug to one patient with the same disease in Africa.	**Rare Diseases matching programme**

***Adapted from ref.52.**

Table 2. Incidence of Aplastic Anaemia in different populations

Population		**Cases per million per year**	**Confidence limits (95%)**
Europe/North America		2	
Thailand		4	
Tanzania	Observed	3.8	2.3-5.9
	'Corrected'	5.9	4.0-8.3

***The incidence of PNH is estimated to be about 5 times lower than the incidence of aplastic anaemia[78]**

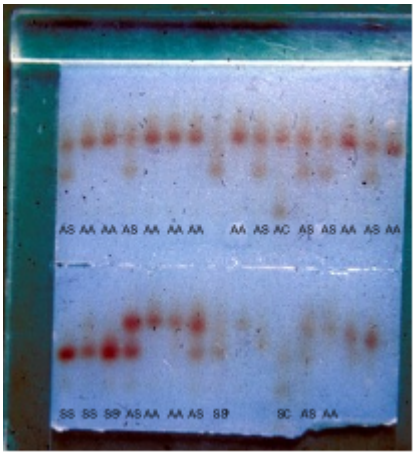

Fig. 1. **Diagnosis of haemoglobinopathies by starch-gel electrophoresis.** The gel could accommodate a total of 32 samples in two rows. The haemoglobin phenotype, that in most cases corresponds to the haemoglobin genotype, is shown in capital letters under each sample. SS[a]: sample from an SS patient who had been transfused. SS[b]: Hb F band prominent (above the Hb S), most likely due to S/β^+ thalassaemia. Samples without letters were regarded as un-interpretable and were subsequently re-run. The cost of the gel was about $4, i.e. about $0.15 per sample (considering the need for re-runs). Courtesy of Vincent C N Okoye, 1971.

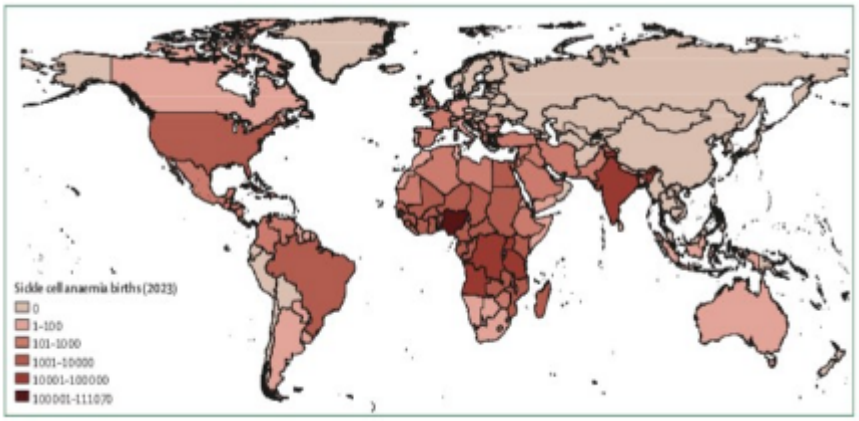

Fig. 2. **Global distribution of sickle cell anaemia in 2023**. Colour shadings illustrate absolute number of births in individual countries.[20]

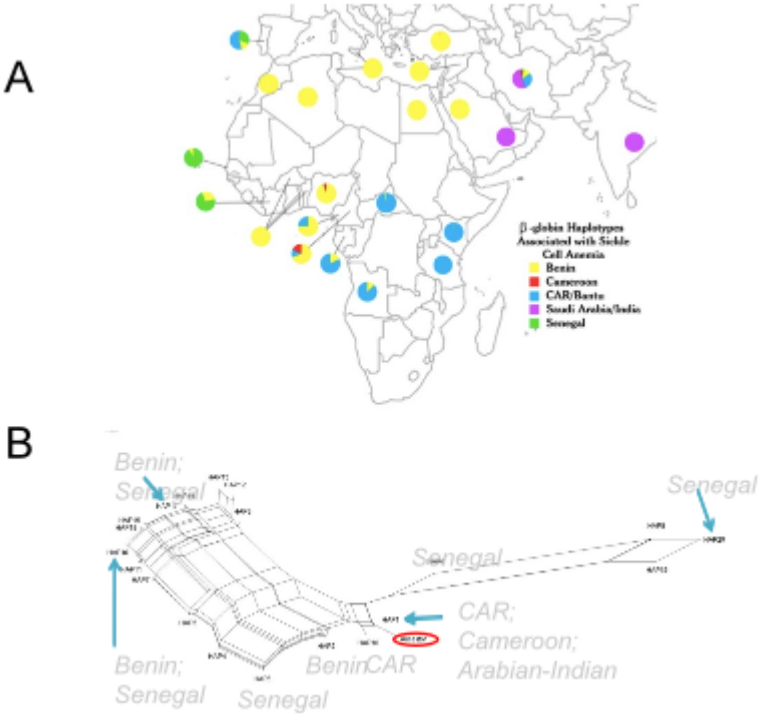

Fig. 3. **Origin of the *HBB*^{E6V} *(sickle)* mutation. A.** Because the mutation is found in the context of different haplotypes in different populations it has been thought that it has originated independently more than once (ref.18). **B.** On the other hand, more extensive sequence analysis seems to be more consistent with a single original mutational event, at least within Africa.[19]

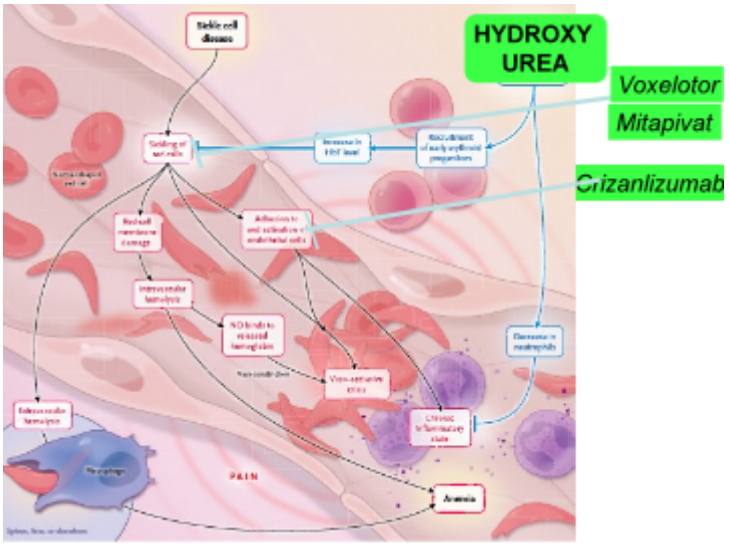

Fig. 4. **A cartoon illustrating the basic pathophysiology of sickle cell disease.** Therapeutic targets of beneficial drugs are also outlined.[26]

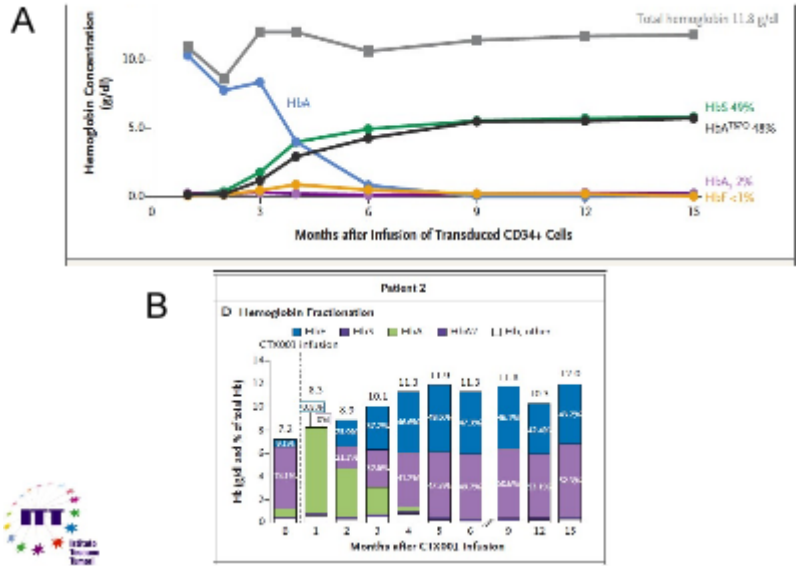

Fig. 5. **Sickle cell disease cured by gene therapy**. **A**. The first patient with homozygous sickle cell anaemia cured by lentiviral-mediated insertion of the normal ♿-globin gene into hematopoietic stem cells: the procedure has essentially converted an S/S homozygote into a phenocopy of an A/S heterozygote.[33] **B**. The first sickle cell anaemia patient treated by gene editing using CRISPR/Cas9 technology. Inactivation of the *BCL11A* gene has caused reversion of the ■🎗♿ globin switch. Thus, within 4 months there is stable production of more than 40% Hb F, with nearly pan-cellular distribution: this makes the patient now a phenocopy of a S/HPFH heterozygote, known to be a benign condition.[34]

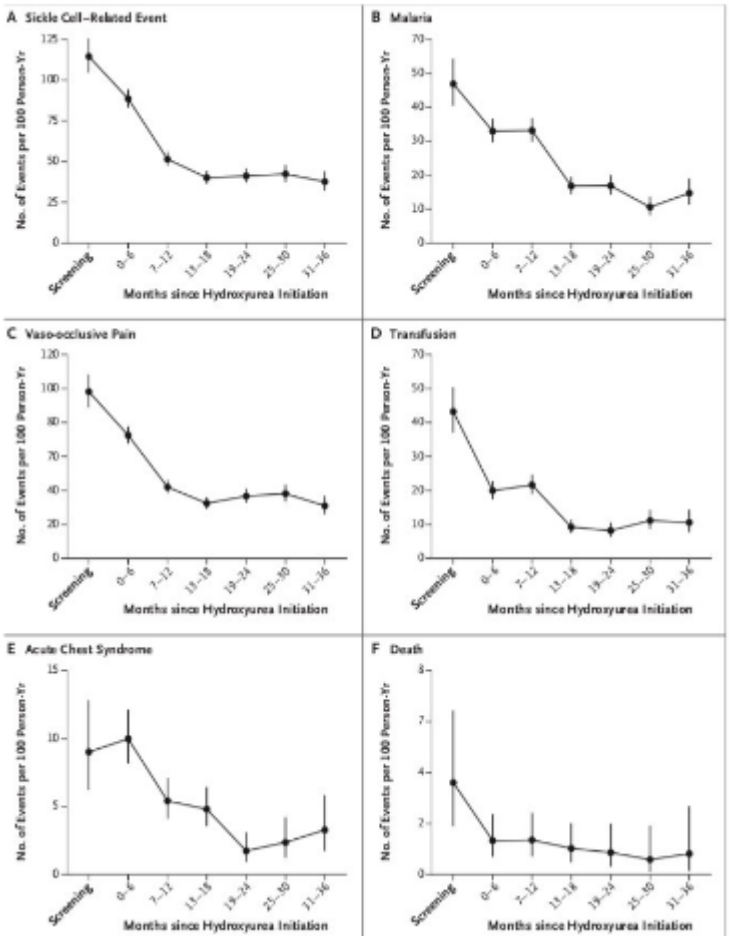

Fig. 6. **Impact of hydroxyurea therapy in patients with sickle cell disease in Africa**. The decrease in malaria episodes was unexpected, but it was subsequently confirmed.[47,48]

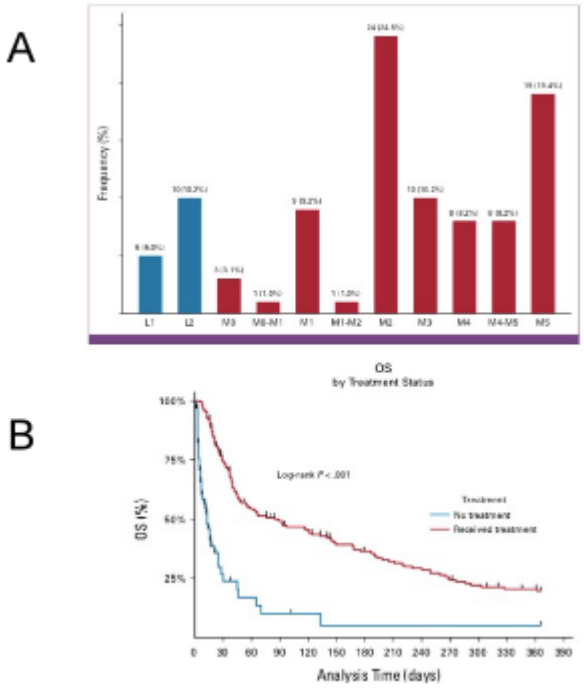

Fig. 7. **Acute leukaemia at the Uganda Cancer Institute, 2009-2018. A.** FAB classification of adults (n = 233). **B**. Overall survival.[54]

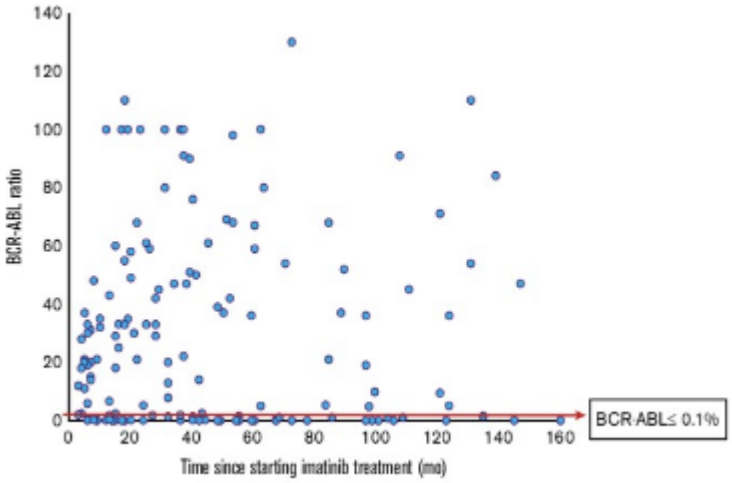

Fig. 8. **The rate of major molecular response to imatinib is rather low in patients with chronic myeloid leukaemia in Tanzania.**[57]

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