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Research today: Diagnostics in the future

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Hippocrates argued that the causes of diseases (or the diagnosis) were physical and could be determined by observing a patient's symptoms. Disease was the result of an imbalance between the four humours or fluids in the human body: black bile, yellow bile, phlegm and blood. Thus, for example if you were lethargic you had too much phlegm and the suggested treatment, citrus fruit (figure 1).

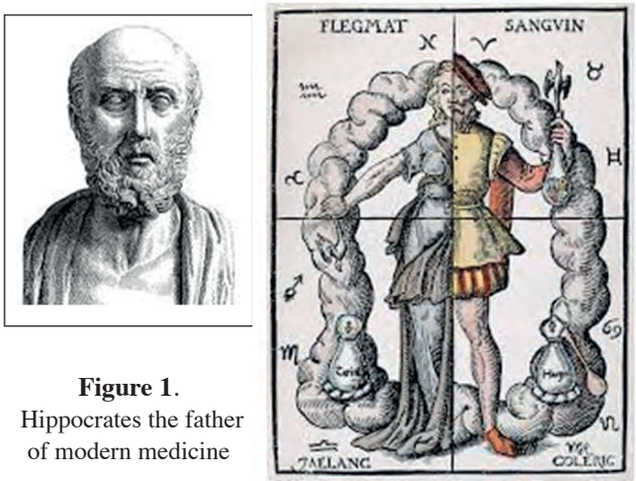

Figure 1.
Hippocrates the father of modern medicine

These ideas of diagnosis and treatment still underpin medicine today.

As doctors we are first of all diagnosticians. I am the doctor in my house and my grown-up children have great faith in me as a diagnostician. From anywhere in the world if you notice any observed symptom or sign you ring Mum for a diagnosis.

Therefore, I have had: "I was out last night and I think I bit my tongue and now my face looks funny". I instruct him to smile at the mirror. "My face is all twisted" he responds. I reassure him "you have a Bell's palsy". The same child aged 25 yrs. rings complaining of an severe pain the chest after playing football and his friends are about to take him to hospital (I am in Portugal, he is in England!).This is followed by a text communication, 'sorry to tell you but they say I am having a heart attack as the heart tracing is abnormal'. I text back: 'You have pericarditis' which was promptly shown to unfortunate attending junior doctor.

And the worst one from a university in England: "Mum I have felt awful and shivery all night and now I have little bruises all over me." I manage to instruct her to urgently get help. She did have meningococcal meningitis (and is fine) but of course as a haematologist I first wanted to know her white cell count and actually had an even worse diagnosis in mind (acute leukaemia).

This is all about making a diagnosis from signs and symptoms. Recognising the pattern and putting it together to come up with the diagnosis.

On entering medical school, we practise the mantra: inspect, palpate, percuss, auscultate and learn to recognise signs and make working diagnoses. Eventually it becomes second nature. (figure 2) In order to develop our examination skills, we have various aids. A prime example is the stethoscope - an

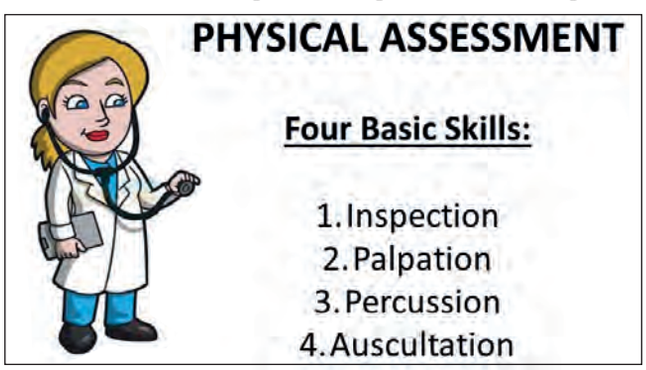

Figure 2. The pathway for detection of signs

aid of the 20th century doctor used to listen to the chest and heart. Interpretation of the findings helps to make a diagnosis.

In former times, the diagnostic process involved careful description of the clinical findings. Ronald Ross in his memoirs describes fever characterised by regular recurrences on a daily basis or every two or three days (quotidian, tertian or quartan) [1]. Starting with chills and followed by high temperatures, the disease was recognised from the pattern of the fever in endemic malarial areas and treatment with quinine was instituted. In the second half of the nineteenth century, with pathological science, the malaria parasite and life cycle was described and to this day in a case of suspected malaria the first step is microscopic examination of a blood film in order to make a diagnosis (figure 3).

Typhoid fever is another example where doctors described the rose-coloured spots and the high fever. Typhoid is one of the

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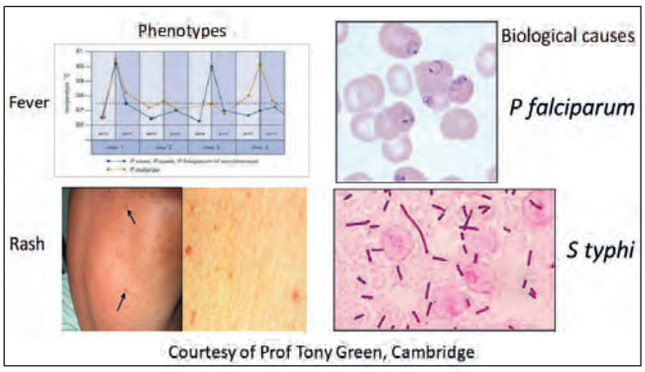

Figure 3. From clinical signs to microscopic pathology in malaria and typhoid.

possible pathogens which killed one third of the population of Athens including Pericles in 430BC and after this disaster the balance of power shifted to Sparta. It was the 1880s before the causative organism *Salmonella typhimurium* was identified. Identification led to an understanding of bacterial transmission and incidents such as the 26-year quarantine of 'Typhoid Mary' who refused to have her infected gall bladder removed.

The diagnostic process proceeds from careful description of the symptoms and signs to formulate possible diagnoses to pathological investigations which reveal the diagnosis.

After entering medicine, I was captured by haematology as a subject when in my fourth year of medical school. I decided that I wanted to be a haematologist and never changed my mind. The attraction of haematology was the mixture of investigative science and medicine leading to a diagnosis. From seeing and examining the patient, the haematologist proceeds to the microscope where the blood is examined and diagnostic findings revealed. I found and have continued to find this fascinating. Not only do you see the patient, take a history and examine them, but the same doctor then observes the blood and the diagnosis may be revealed.

This process is seen going back to the nineteenth century. John Bennett was reputed to be the first person to describe leukaemia as a blood disorder in 1845 (although Virchow published similar results 6 weeks later). John Bennett in 1845 at the age of 33 was already a fellow of the Royal Society, although he was described as 'a man of brilliance but short temper, certain of his own virtues, pugnacious and unable to suffer fools'. He described the microscope findings in a very sick patient. There appeared to be huge numbers of colourless corpuscles (or cells) which resembled pus. In those days no counterstain was used when looking at a blood film so cells did appear colourless and he was looking at the large numbers of white cells seen in a patient with the white cell proliferation of leukaemia [2]. This was a case of chronic myeloid leukaemia.

Following this initial description, leukaemia was subtyped and defined by the microscopic findings as were many other haematological disorders.

Acute myeloid leukaemia was subclassified by the FAB group (French/ American/ British) in 1976 where a number of the 'great and the good' in haematology sat round a multi-headed microscope looked at a large number of cases and divided them into seven different subgroups depending on the morphological appearance [3]. Acute promyelocytic leukaemia or the subgroup M3 is perhaps the clearest example of this. There is a definitive picture of heavily granulated promyelocytes where the distinct morphological appearance defines the subtype.

There are many other examples of the use of the microscope to arrive at a definitive diagnosis. One fascinating piece of research in a different area is that of peptic ulceration. When I was a junior house officer in surgery in the Mater hospital in 1980, we had a surgical ward full of people who had had major invasive surgery for duodenal ulcers. Medical therapy in the form of H_2 blockers and later protein pump inhibitors initially came along that year. However, it was the work of Barry Marshall and Robin Warren for which they won the Nobel prize in 2005 which shows that *helicobacter pylori* infection was the cause. Barry Marshall drank *H. pylori* and developed symptoms of peptic ulceration within 5 days and had inflammation and *H. pylori* in his stomach. This linked the long described clinical findings with the causative organism.

In haematology, the process of linking clinical findings and patterns in the blood picture to define disease continued. In 1951 the preeminent American haematologist William Dameshek published a very short paper describing and identifying the myeloproliferative diseases. These were individuals with elevated red cells, white cells and/or platelets. He described these as polycythaemia vera (PV), when the red cells were primarily the issue; essential thrombocythaemia (ET) when it was the platelets and chronic myeloid leukaemia when it was primarily a white cell problem [4]. To further define these, complicated clinical and laboratory criteria developed which had to be fulfilled to make a diagnosis of PV or ET.

Included in this classification were those with who appeared to have proliferating white cells, termed chronic myeloid leukaemia. This diagnosis was clarified over subsequent decades as new investigation revealed more of the malignant process. Chronic myeloid leukaemia is characterised by a markedly raised white cell count and a packed bone marrow, full of white cell precursors. Cytogenetic investigation reveals a small chromosome 22 in these patients, the so called 'Philadelphia chromosome' because it was initially described in Philadelphia. Over the decades this chromosomal change was dissected at the molecular level where the reciprocal translocation between chromosomes 9 and 22 takes place leading to a new fusion gene, *BCR-ABL*-1. This leads to a fusion protein made from the new gene (Figure 4). This abnormal protein (tyrosine kinase) drives the disease. However, the discovery of the disease pathway led on to a definitive treatment. A drug to block the tyrosine kinase protein was developed and a number of tyrosine kinase inhibitors are now available [5].

Sir John Dacie was considered to be the father of British

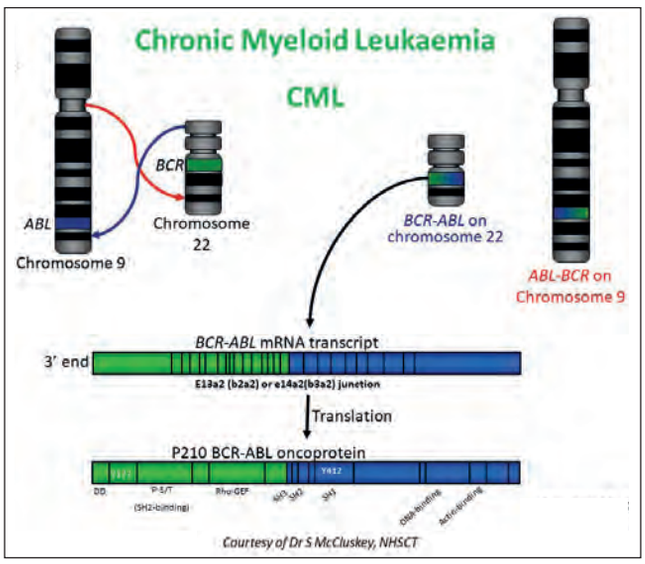

Figure 4. The *BCR-ABL-1* reciprocal translocation of chromosomes 9 and 22 in chronic myeloid leukaemia

haematology. I went to work in the Hammersmith hospital (Royal Postgraduate Medical School) in 1989 as a senior registrar. Sir John Dacie was occasionally still around and a great influence. One of the things that he and colleagues had described world- wide was the rare and fascinating disorder paroxysmal nocturnal haemoglobinuria (PNH) where rare patients were seen who produced red urine in the early morning and had devastating haemolysis. Extensive laboratory studies showed that these patients' red cells lysed in acidified serum along with other complicated patterns of biochemical abnormality. However, Sir John Dacie and his collaborators have been described as 'stamp collectors'. They collected rare cases and studied them in great depth clinically and in the laboratory and recognised patterns of disease making diagnostic groups.

In the 1980s and early 90s the molecular lesion was described in individual cases of PNH where mutations were seen in the *phosphatidylinositol glycan Class A* (*PIG*a) gene. This mutated gene produced a mutated protein on the surface of the red cell which does not function normally. This results in failure of binding of other proteins to the red cell surface. Therefore, the red cell becomes unstable in the presence of complement and the cell lyses resulting in catastrophic haemolysis. Thus, in PNH the diagnostic pathway began with careful clinical description but it is with molecular diagnostics that the disease mechanism is explained. Understanding of the molecular lesion led to the development of a treatment to control the haemolysis. Eculizumab, a humanised monoclonal antibody specific for the human plasma component C5, binds to it and thus blocks the complement pathway. This blockage of complement activation protects the red cell from complement-mediated lysis and stops haemolysis [6]. The pathway here is from clinical description, to accurate molecular diagnosis to effective treatment.

Another haematological example as to how diagnosis developed is one of the types of acute myeloid leukaemia described on morphological appearance by the FAB group. This is acute promyelocytic leukaemia, M3, the one with the obvious morphological appearance with large numbers of these needle-like structures (Auer rods) in the promyelocytes. This disorder was recognised by this appearance and classified on this basis by the FAB group. The clinical pattern of disease with patients who were seen to have a bone marrow full of this type of blast cell often presenting with catastrophic bleeding. Further investigation revealed that the patients with disease with this morphological appearance had a particular chromosomal abnormality, a t(15;17) translocation. The molecular lesion associated with the translocation was discovered. The *promyelocytic leukaemia* (*PML*) gene and the *retinoic acid receptor alpha (RARA*) gene was found to be fused together and this new fusion gene produces an abnormal protein which drives the disease. Explaining this mechanism led to treatment with all- trans-retinoic acid (ATRA) which leads to blockage of the fusion protein reducing the incidence of life threatening bleeding and ultimately chemotherapy treatment of the disease [7]. Full diagnosis therefore leading to effective treatment.

Similarly, with acute myeloid leukaemia, cytogenetics and molecular diagnostics have led to much greater understanding of acute myeloid leukaemia with many different subgroups defined on a molecular basis. This has had many iterations and expansions since the original 1976 description of 6 subgroups defined on the basis of the microscopic picture only. The 2016 WHO classification of acute myeloid leukaemia extends for many pages with some different types defined on the basis of recurrent genetic abnormalities [8]. Current work is addressing the issue of finding specific therapies effective against all of these genetic abnormalities leading to 'precision medicine' where there is a specific drug for each molecular lesion. However, there remain many types where the genetic lesion is not yet defined.

In my area of interest, the myeloproliferative neoplasms molecular genetics have taken classification a lot further that the original definitions originally formulated by Damashek [4]. In 2005 it was discovered that many patients with myeloproliferative neoplasms had a single point mutation in the *Janus kinase 2* or *JAK*2 gene. The amino acid at position at point 617 is highly conserved across species from bacteria to man suggesting it in highly important functionally and this proves to be the case (figure 5). When the amino acid is changed it leads to a constitutively activated protein on the receptor which signals even in the absence of a driver ligand. This ultimately results in increased cell production and as the JAK protein is on multiple receptors it would account for increases red cell, white cell or platelet production as seen in the myeloproliferative neoplasms [9].

The discovery of the *JAK*2 mutation and then subsequently mutations in *myeloproliferative leukemia virus oncogene* (*MPL)* and *calreticulin (CALR)* genes meant that diagnostic criteria for polycythaemia vera and essential thrombocythaemia could be simplified and clarified. However, these discoveries give rise to further questions. Is the presence

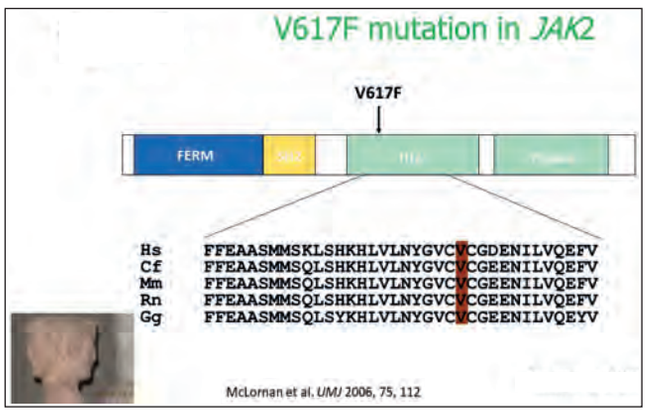

Figure 5. Mutation in the *Janus Kinase* 2 gene is highly conserved across species

of a mutation and an abnormal blood count enough to make a diagnosis rather than the previous diagnostic criteria based on a description of phenotype alone? What other factors, genetic or otherwise, influence the ultimate diagnostic picture and need to be considered in the diagnosis? And is it valid to split up disease into these different subgroups or is the molecular detection of an acquired clone indicative of a unified diagnosis?

These are some of many examples where the diagnostic process has proceeded from clinical description, investigation often microscopically to molecular definition which then leads to development of effective treatments. However, there are other clinical situations where there is nowhere near this degree of diagnostic definition. Many of my haematology patients complain of a lot of aches and pains. They tell me they have fibromyalgia. NHS websites give criteria for diagnosis as severe pain in 3 to 6 different areas of the body or milder pain in 7 or more areas with symptoms at a similar level for at least 3 months in the absence of any other explanation for the symptoms[10]. That is very non-specific. Surely, a much better diagnostic test is needed to classify and understand the phenomenon.

Another example seen microscopically is the phenomenon of haemophagocytosis where there is consumption of cells seen in the bone marrow and other organs. In this haemophagocytic lymphohistiocytosis, a rare immune disorder, the body reacts inappropriately to a trigger usually infection. The microscopic phenomenon of haemophagocytosis is associated. In children this reaction is associated with specific genetic abnormality but in adults it is now described with many different triggers. Diagnostic criteria are complicated and convoluted. Extreme hyperferritinaemia is considered to be a marker but this is a very non-specific test [11]. Recognising the disorder early and attempting treatment are required given the catastrophic clinical course and the high associated mortality. However, the pathological process and triggers of that process in individuals are not really understood. More definitive and specific diagnostic tests are needed to sort out these presenting signs and symptoms.

Having seen how the diagnostic process has developed and evolved over time, I would like to consider how current and future developments may aid the evolution of diagnosis although of course this will always be speculative.

It is interesting to reflect in general how technological development changes revolutionise our world. In little over a hundred years the motor car has gone from a new but revolutionary means of transport to the object which every young person aspires to on reaching 17 years of age. In the future cars will certainly be electric and may be something to hire or rent whenever we have need. The telephone similarly has transformed from an object present only in the houses of the rich to the ubiquitous small device from which we cannot be parted and of which we only use a tiny fraction of the available computing power at any time. There are many other examples of technological advances which change how we navigate our lives, such as moving from paper maps to the GPS (global positioning system) device on mobile phone as the only way to get around (or diagnose how to get to a destination !).

In medicine new methods are rapidly changing the diagnostic process. The stethoscope has been central to a physician's identity. The stethoscope of the 21st century is point of care ultrasound (POCUS) which enables us to look rather than listen and the patient can share the experience. Use of the ultrasound will become a core competency for physicians [12]. Imagine a future where you have a small ultrasound machine in your pocket instead of a stethoscope.

Returning to the microscope, with computer technology many thousands of cells can be rapidly examined , patterns recognised and cells classified without the need for a person to look and count. This raises a point about which many haematologists are passionate. When will the microscope be obsolete? With the microscope a phenotypic diagnosis is made but with computers scanning and evaluating patterns in vast numbers of cells will this replace the need for simple morphology? Whereas we are all trained in the use of the microscope is the time coming when this is unnecessary and machines will be able to make the diagnosis.

The future of diagnostics will embrace molecular genetics. These tests are now part of the routine workup. No matter what the area of medicine, we look to the genetic code for the diagnosis, either by using specific next generation sequencing panels aimed at looking for specific defects or perhaps in the future more frequently investigating the whole genome. This can be seen in a recent study looking for clonal defects in blood samples from normal populations. The number of recurrent somatic mutations or clones detected increases exponentially with increasing age [13]. This is termed age-related clonal haematopoiesis (ARCH) but what does it mean in diagnostic terms. Will these clones develop into malignant disease? Surely not in all cases and is there any treatment to supress the clonal disease?

Computer assisted data collection of clinical and laboratory information and pattern recognition is now occurring on a grand scale. This is the same process as our early

'stamp collecting' haematologists but with vastly more power. Country wide collection of clinical, laboratory and molecular data is in the planning process and this will give an enormously rich source for evaluation. This leads to the development and use of algorithms where large amounts of data are processed and then used by following algorithms to make a diagnosis and then one step further to artificial intelligence. Can the analysis of large amounts of data and its use in diagnostic algorithms improve the making a diagnosis?

Will new and better pattern recognition lead to clearer diagnostic classification ?

Better diagnostics are crucially important as this leads to better treatment. The example of chronic myeloid leukaemia where the understanding of the diagnosis led to the development of a tablet to treat the disease and the patient diagnosed with CML today would expect to have a similar life expectancy to their age and sex matched peers [14].

However, that is not the case with many of the diseases treated today. We regard our forbearers who bled and purged patients in order to get rid of the 'Bad Humours 'as administering barbaric treatment but perhaps much of what is done today in treating malignancy will appear equally barbaric in the future. In haematology we treat leukaemias by destroying all bone marrow cells with chemotherapy and then try to keep the patient alive through infection and bleeding waiting for the normal blood cells to grow back. Perhaps with more accurate diagnosis in the future, better and much more targeted treatment would avoid such blunderbuss management.

The development on new diagnostics is an ongoing process but as I write up this inaugural lecture in the middle of the COVID 19 pandemic, astounding advances have been incorporated into practice in an astonishingly short period of time. Practice has rapidly switched to online and telephone consultation and we have adapted to changes in practice which would have seemed impossible just a very few weeks ago. There are many technologies available which have facilitated the changes in our practice.

And it is with this in mind I invite you to consider fundamental and clinical research and to think how diagnostics in the future will lead to new and more precise diagnosis.

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