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Opinion

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Is Polycythaemia Vera a maternally transmissible disease ?

ABSTRACT

The hypothesis that cancer is caused by mutations in driver genes is widely accepted, however the theory depends on the mutations occurring spontaneously. In patients with the disease Polycythaemia Vera it has become common to perform a test for the presence of a specific driver-mutation that leads to the amino acid substitution p.V617F in the *JAK2* protein. Hitherto, this mutation has been considered to be the result of a spontaneous mutational event occurring frequently in the same nucleotide base in each person with the disease. But with increased understanding of the biology of mutations, there is the need for a more plausible hypothesis in respect of the causation of Polycythaemia Vera. Considering the disease as being a clonally transmissible disease passing from a pregnant woman to her unborn child would appear to give an explanation of the observed facts. However, laboratory confirmation is needed to confirm this is indeed the case. The implication of a disease being transmissible in this manner raises important questions.

Keywords: haematopoiesis, *JAK2*, JAK2 V617F, polycythaemia vera, essential thrombocytothaemia.

Introduction

Over the last few months a number of papers have appeared that deal with the subject of cancer-driver genes (Bailey, 2018; Thorsson, 2018) and these have largely been associated with publication of The Cancer Genome Atlas (Tomczak, 2015). The Atlas now gives details of 299 proposed cancer-driver genes. However, the mechanisms that underlie how mutations in chromosomal DNA appear and cause cancer are unclear; and certainly there is no unifying theory to explain the steps involved. In this opinion paper the evidence for just one mutation in a cancer-driver gene causing a particular disease is examined step-by-step and a hypothesis suggested that might apply to this disease; and quite possibly to other conditions, such as Essential Thrombocythaemia.

Polycythaemia Vera (PV) is not a particularly common disease, but its existence is well recognised. However the condition of PV shows several enigmatic features; and although a lot of research has been done into the condition the aetiology remains uncertain. Here a hypothesis is put forward suggesting the basis of PV is a maternally transmitted disease with clonal cells being passed *in utero* to the foetus, which may, or may not, lead to the development of overt disease during the lifetime of the recipient.

The clinical disease

In PV there is an excessive production of red blood cells and the presentation of the disease can be non-acute, acute, or as an incidental finding following a routine medical examination. In most non-acute patients there are no particular features in the history or clinical examination to suggest the diagnosis, however typical complaints from patients are of tiredness, a general feeling of ill health and perhaps the noticing of a flushed appearance of the face and a blueness of the hands. A

subsequent blood sample showing a raised haemoglobin level and an above average number of apparently normal red blood cells will suggest the diagnosis of PV; but a formal diagnosis is often made only after further tests confirming the overproduction of cells. In acute cases the presentation can be with a heart attack, stroke or other thromboses; and the diagnosis made following blood tests. However, nowadays, more and more instances of the disease are being diagnosed following routine blood tests, but in these patients the disease is likely to be at the early stage and simple observation rather than any more aggressive treatment may be appropriate (Spivak, 2002; Callum, 2008).

It is difficult to give an accurate figure for the frequency of PV as cases are not reported, but the clinical condition may affect as many as 1 person in 10,000. However, if the early symptomless cases are included a figure nearer to 1 person in 2,000 may be more accurate. The condition appears to affect more men than women and a diagnosis is usually made in people of about 50-60 years of age (Grunwald, 2018).

The rise in the number of circulating erythrocytes leads to an increase in the viscosity of the blood and thereby an increasing liability to thromboses affecting the heart, brain and other organs. So undiagnosed and untreated PV can be serious and early detection can be life-saving (Kroll, 2015). In most instances medical treatment is given to people diagnosed with PV with the aim of returning the blood count and viscosity to near normal levels and thereby decreasing the risk of thromboses. For many decades phlebotomy has been the standard treatment with usually 500 mL. of venous blood being removed from the patient at regular intervals, such as weekly initially and perhaps only every few months afterwards. The risk of thromboses can also be reduced by low-dose aspirin taken daily (Grunwald, 2018). With treatment PV is often relatively symptomless for some years and for many people PV can be an unimportant condition. However, in some the disease requires more active intervention and treatment with Hydroxyurea, Interferon and full anti-coagulation may be necessary. But as the condition does not remit, constant monitoring is essential.

If a patient, or their advisor, wishes to learn more about the condition, they will usually be told about several of the features that make PV an enigmatic condition. Such as: the extra red cells appear to be quite normal and show no abnormal features; PV appears sporadically and for no apparent cause; it is not a chromosomal, nor mitochondrial, inherited disease and the extra red cells appear to derive from a single clonal stem cell; the cell line is relatively unresponsive to the action of the hormone erythropoietin; a marker for the disease has now been identified on chromosome 9 in the *JAK2* gene; and most importantly there is continuing confusion over whether, or not, PV is a cancerous condition.

The existence of a genetic marker for PV has radically changed the way in which the disease is now diagnosed and a positive test result will often be taken as confirming the presence of the disease. A report might be given in a similar form to: *the mutated V617F was detected in heterozygosity in the JAK2 gene* (personal communication and translated from the Portuguese original). However, such a test does not appear to be of any prognostic value; and it is no means certain that a positive test in a symptomless person will lead to clinical disease appearing within their lifetime. Moreover, the survival of a person diagnosed with PV, perhaps by just by having a slightly altered blood count, a positive JAK2 test and being below the age of 60, may now be as long as 24 years (Tefferi, 2018). But whilst a positive result

may be considered accurate, a negative result is not so reliable because of the technical difficulties involved in performing the test (Hinds, 2016).

In the following discussion these points will be addressed in turn and the hypothesis suggested that PV is maternally transmitted.

Discussion

The first person to describe the condition later to be called Polycythaemia Vera was the well-respected Parisian doctor Louis Henri Vaquez, who in 1892 wrote *Sur une forme spéciale de cyanose s'accompagnant d'hyperglobulie excessive et persistante,* which nowadays might have been given the title '*A case of cyanosis caused by a sustained increase in haematopoiesis*'. A few years later the famous Canadian physician Dr William Osler also described the condition and pointed out that there could be an enlargement of the spleen; and for some years the name Osler-Vaquez disease was in use (Strieff, 2002).

There was no real progress in the understanding of the cause of the disease over the next 60 years as shown by the report of Dr Louis R Wasserman, Chairman of the newly formed *Polycythemia Vera Study Group*, to the 1968 meeting of the American Society of Hematology (Wasserman, 1968). His paper concentrated mainly on the range of therapies available at the time, which were phlebotomy, and in resistant cases, the use of cytotoxic drugs and, in particular, of radioactive ^{32}P which, although helpful, was perhaps leading to an increase in leukaemia cases.

[Obituary available at: https://www.nytimes.com/1999/06/23/nyregion/dr-louis-r-wasserman-88-authority-on-blood.html]

The first real development came some years later when it became understood that PV was a clonal disease. And a research team described PV as being caused by *an unknown somatic stem cell defect that leads to clonal myeloid hyperproliferation*. Their report also suggested the disease perhaps showed *an autosomal dominant inheritance pattern with incomplete penetrance* (Kralovics, 2003).

Another feature of PV that emerged at a similar time was the finding of a lack of responsiveness of the clonal cells to changing levels of the newly characterised hormone, Erythropoietin. The existence of a hormone that controlled the production of red blood cells was suspected from early in the 1900's. But it was not until 1977 that useful amounts of purified hormone became available (Miyake, 1977). Erythropoietin promotes the production of red blood cells, and is part of a feed-back loop. In PV cases the levels of the hormone are characteristically very low, as might be expected; but the clonal cells continue with the excessive production of the red blood cells despite the absence by Erythropoietin (Remacha, 1997).

The next major step forward took place in 2005 when several research groups published similar results showing that PV was in some way linked to a mutation in the *JAK2* gene (Vainchenker, 2005). For example the group from Switzerland writing in the New England Journal of Medicine reported in April of that year: *The frequency of V617F was 65 percent among patients with polycythemia vera* (Kralovics, 2005). And hence forward testing for what has been termed **JAK2 V617F** has become an integral part of making a diagnosis of PV. Indeed the World Health Organization makes the presence of a *JAK2* mutation a major criterion for the diagnosis of PV (Arber, 2016).

But what exactly is meant by being positive for **JAK2 V617F** ?

The nucleotide base on chromosome 9 with the coordinates 9:5073770 is normally a 'G' (for guanine), but if this changes to a 'T' (for thiamine) the resultant amino acid produced in the JAK2 protein changes from V (for valine) to F (for phenylalanine); and as this is the 617th amino acid in the JAK2 protein the mutation is for simplicity described as **JAK2 V617F.** This mutation may also be described using the Reference Single Nucleotide Polymorphism cluster ID (SNP) **rs77375493.**
[https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=77375493]

However what is not often appreciated is that this mutation only affects the chromosomal DNA of the cells in the proliferating stem cell clone found in PV cases, and not the chromosomal DNA of the other cells of the body. Therefore, the testing for **JAK2 V617F** detects the presence of the mutated DNA in nucleated cells of the stem cell line (but not the red blood cells as these do not retain their nuclei) and in any free chromosomal material. The result of the test is more accurately given as a percentage of mutated DNA as compared to the amount of non-mutated (so called *wildtype*) DNA, rather than just a simple *positive*, or *negative* (Xu, 2007; Hinds, 2016).

The present accepted explanation of the aetiology of PV is that the mutation **JAK2 V617F** acts as a 'driver mutation' that occurs *spontaneously* in a single stem cell in the haematopoietic system (Callum, 2008; Kent & Green, 2017, Rumi & Cazzola, 2017). A clone of cells then develops with this mutation in their chromosomal DNA. As the mutation gives a 'gain of function', the cells are able to continue to multiply irrespective of the low level of Erythropoietin; and over time the red blood cells produced slowly increase as a proportion of the total red cell population. This results in increasing viscosity of the blood and the possibility of thromboses (Kaushansky, 2005).

Opinion

The main question to answer concerning the aetiology of PV is whether it is possible for the mutation c.9:5073770G>T to occur *spontaneously* in stem cells in the haematopoietic system in each and every person of a large number of people. Clearly as this mutation is very rare in normal DNA, the suggestion that the mutation can occur frequently *de novo* is far from being a plausible explanation. For a mutation to appear repeatedly at the same point in a gene there would have to be a structural weakness at that point, leading to a break in the DNA strands and a specific type of mutation, here the unusual transversion G>T, occurring as the DNA is repaired (Wei, 2007). But as the mutation c.9:5073770G>T is rarely seen in the DNA of normal cells, this would indicate there is nothing inherently unusual about the nucleotide sequence of the gene and accordingly no structural weakness, or other reason for the mutation to occur at that particular point.

It follows if the mutation does not occur *spontaneously* then the copy of the *JAK2* gene with the allele c.9:5073770T must be *acquired* in some other way; which means there has to be a copy of the mutated gene in existence. The accepted means of *acquiring* a particular trait are through chromosomal, or Mendelian, inheritance, or by transmission; and in respect of PV as there is no inheritance of the allele c.9:5073770T, it only leaves transmission as the means by which the mutated gene is *acquired*.

Three types of transmission need to be considered: (1) the possibility PV is acquired as a *transmissible cancer*; (2) by transmission from person-to-person; and

(3) by vertical transmission from mother-to-foetus.

(1) *Transmissible cancer*: although this type of transmission is known to occur in some animals, there have not been any examples found in humans. The most studied *transmissible cancer* is seen in dogs where the neoplastic condition of Canine Transmissible Venereal Tumour (CTVT) is considered to be a clonally transmissible cancer (CTC). This condition has been researched extensively for many years and numerous papers have been produced. Two papers in particular (Murchison, 2014, Frampton, 2018) give a detailed analysis of the disease, its progression and the response to therapy. CTVT lesions usually grow on the skin of a dog, especially on the external genitalia, and are transmissible by licking, biting and mating. Presumably the tumour is spread by viable tumour cells from an affected animal entering the skin of other animals through abrasions or larger wounds. The lesions can be multiple and metastatic, although metastatic spread would appear not to be common. The disease was first described over 200 years ago and has a worldwide distribution.

A second CTC is Tasmanian Devil Facial disease (DFTD). The Devil is found only in the wild on the island of Tasmania. This small carnivorous marsupial mammal was fairly numerous in the past, but the population has fallen rapidly in the last 100 years and particularly so in the last 30 years. The Devil is now an endangered species; and the presence of DFTD may lead to the animal's extinction within a few years. DTFD is transmitted from one Devil to another by bites. The disease is characterized by the development of a single or multiple lesions in and around the mouth and these lesions can grow to a large size, ulcerate and lead to the death of the animal (Murchison, 2008, Ujvari, 2013, Pye, 2016).

These two examples show that the transmission of a CTC is possible by direct contact of an affected animal with another (Greaves & Hughes, 2018). But the most distinctive feature of any cancer is the way in which it produces cells of many different shapes and sizes, none of which have normal function. So PV cannot be considered to be a *transmissible cancer* as the PV clonal cell line appears to have cells with normal behaviour and has the ability to produce competent red blood cells. The point of PV having normal cells does bring into doubt the question as to whether the disease should ever be considered as a cancer; or even as a pre-cancerous condition.

(2) The second method transfer to consider is *person-to-person transmission*; where a person with PV might be able to pass the disease on to another person. This type of transmission is, of course, commonly seen when considering the aetiology of viral, bacterial or protozoal infections, and the prion based *Creutzfeldt-Jakob* disease, but the transfer of a harmful allele in the chromosomal DNA is another matter; and there are no proven examples.

So can PV be transferred from one person, with quiescent or overt disease, to another person who does not have the condition; and it would appear that this should be considered carefully. But it does appear unlikely because of a number of factors, such as: the occurrence of PV does not appear to be related to the use of blood transfusions, or other ways in which blood might be passed from one person to another; the incidence of PV does not appear to cluster in geographical areas or in particular groups; and, most importantly the innate immunity system attacks non-self-protein (Billingham, 1953; de Boer, 1987). As a result of these factors it would therefore appear unlikely that *person-to-person transmission* of PV occurs in healthy and immunocompetent people.

It is interesting to point out that in most instances when blood has taken from the person with PV by phlebotomy, this blood is not offered for transfusion; although the evidence for refusing to use the blood is somewhat anecdotal. However the increased viscosity of the blood may be enough to limit its use.

(3) The third method to consider is *vertical transmission from mother to foetus*; and this would appear to be possible. Exchange of cells between mother and foetus does occur during a pregnancy; and in particular, the passage of foetal cells to the mother is well documented and, as an example, forms the basis of maternal blood testing for trisomy 21 in the foetus (Lo, 1998; Kasemi, 2017). However, of the transfer of maternal cells to the foetus is less well documented, however the subject has been reviewed recently in some detail (Greaves, 2018).

Vertical transmission would imply that stem cells carrying the mutation **JAK2 V617F** pass from the mother through the placenta to the foetus and successfully establish themselves in the foetal bone marrow. Subsequently, the stem cells may form a clone; but the development of clinical PV will only occur if there is a subsequent excessive production of red blood cells. If the foetus is male, no further transmission will occur, but if female further transmission to the next generation will be possible in due course of time.

The chances of transmission happening in this way presumably would depend on the size of the PV clone in the mother at the time of the pregnancy and as the size increases slowly with age, it may be expected that PV will show a *maternal age effect*. This means that a person born of an older mother possibly has a higher chance of developing PV, than a person born of a young mother; and indeed the age of the maternal grandmother may also be significant. At present the effect of maternal age is really only observed on the frequency of trisomy 21 where there is a marked increase in risk when a mother is over the age of 40 (Kasemi, 2017).

At present there is no laboratory evidence giving confirmation that *vertical transmission* is the basis of the aetiology of PV and research will be required to confirm if this is indeed the case. In this respect it is useful to consider a brief report in which it would appear that vertical transmission was seriously considered, but the conclusion made at the time was that the **JAK2 V617F** mutation had been 'somatically acquired *in utero*' (Langabeer, 2013). However, a study into a possible *maternal age effect* would be comparatively simple; and it is a pity this factor was not considered in a recent project (Hinds, 2016). It is also interesting to consider that mitochondrial haplogroups are passed from mother to child; and it may be possible to identify correlations between particular haplogroups and people with PV. Again this possibility was not explored in the research project, although the information was available (Hinds, 2016)

Cancer v. non-cancer: the question as to whether, or not, PV is a cancerous condition has been a point of discussion for many years (Spivak, 2002). But the World Health Organisation has come down clearly on the side of cancer by describing PV as one of a number of myeloid neoplasms (Arber, 2016). The main reason for this decision would appear to be because the mutation **JAK2 V617F** is considered a driver mutation. However, an important feature of PV is the absence of any neoplastic cells. Therefore it would seem sensible to tell patients that, of itself, PV is not a cancer.

PV frequency: It is difficult to give a definite figure for the incidence of PV as the disease is not reported and the available figures vary widely. In 2008 the

incidence was said to between 0.6-8.7 per 10,000 and 2 in 100,000 (Callum, 2008); but it is unclear that figures were strictly comparable. Overall, an incidence of 1 person in 10,000 with clinical disease might appear appropriate. However, the disease is becoming diagnosed more frequently nowadays because of the ageing population, routine blood tests being more frequent, and the fact **JAK2 V617F** testing is becoming a part of routine practice when an abnormality is found in an initial blood count (Grunwald, 2018). A report from China has suggested that the **JAK2 V617F test** may be positive in almost 1% of the population (Xu, 2007); therefore it is possible that there are many people who might develop clinical signs and symptoms of PV when they get into older age. The tendency for women to be having pregnancies at an older age may also lead to an increase in PV cases, but this will only occur if there is indeed a *maternal age effect*.

Conclusion

The hypothesis that a *somatic*, or *spontaneous,* mutation explains the aetiology of PV is very simplistic and with increasing knowledge of the biology of mutations this is no longer a plausible explanation. Here, a hypothesis is presented that suggests that *vertical transmission from mother to foetus* of clonal stem cells may transfer the **JAK2 V617F** mutation from one generation to the next. If so, this type of *transmission* in PV may be just the first example of this type of inheritance; and this mechanism may apply to other diseases. The aetiology of cancer is poorly understood and the hypothesis presented here may help just a little towards an understanding of the significance of individual driver mutations. In addition, the suggestion that both maternal age and mitochondrial haplogroup may be important epidemiological factors in PV should be considered further.

Conflict of Interest. The author declares that he has no conflict of interest.

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