Review

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Review

The Journey to Clonal Hematopoiesis and Applications in Cardiovascular Disease and Cancer

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Abstract: For a long time, atherosclerosis has been regarded as a mere lipid deposit in the blood vessels. However, in the recent years a growing body of experimental and clinical evidence have highlighted the role of inflammation and immunity as a central mechanism of disease. Moreover, in the last decade the coming of next-generation sequencing and its application to large human population has broken the barrier between inflammation and cancer. Indeed, acquired mutation in key genes related to the control of hematopoiesis and myeloproliferation have paved the way to establish the new concept of clonal hematopoiesis of indeterminate potential. This phenomenon is being considered not such "indeterminate", but as an emerging cardiovascular risk factor. Thus, this may explain the mechanisms of myeloproliferation and inflammation in atherosclerosis. And in a bidirectional journey, it has helped to explain the extremely high cardiovascular risk in cancer survivors, in particular in myeloprolfierative neoplasm patients. A deeper understanding of these mechanisms may pave the way for the future early diagnosis and potential preemptive treatments of the leading worldwide cause of death.

Keywords: atherosclerotic; inflammation; bone marrow ; clonal hematopoiesis ; myeloproliferative neoplasm; autoimmunity; cardiovascular risk; cardio-oncology

1. Background: From Atherosclerosis to CHIP

1.1. First Steps

In our everyday life, there is a clear conceptual distinction between what is cancer and what it is not cancer. However, the identification of chronic inflammation as a putative cancer trigger is not new, since the sum of many inflammatory events may eventually lead to a pre-neoplasic and eventually a neoplasic lesion [1]. Given that in last decades it has become increasingly evident that atherosclerosis is an inflammatory disease where innate and acquired immunity both trigger and amplificate the disease [2], the question arises why this chronic inflammation does not lead to cancer. Or is it the case?

The history of the research on robust cross-talks between cancer and inflammation, in particular atherosclerotic vascular disease, takes us to the 19th Century, when Virchow first established the link between inflammation and cancer, as well as the link between inflammation and atherosclerosis [3]. Moreover, a century after, in the 70s of the 20th Century, Benditt and Benditt identified a monoclonal origin in vascular smooths muscle cells from atherosclerotic lesions [4]. However, these studies were performed in a time when vascular smooth muscle cells were considered as the key cell for atherosclerosis, rather than blood cells. Blood was going to emerge and the key tissue to look at in the coming decades. In the meanwhile, a strong conceptual framework was built between atherosclerosis and inflammation the by concurrence of vascular smooth muscle cells, endothelial cells, leukocytes and platelets. This complex interaction was considered as the basis of the injure response theory, which was considered as the mainstream atherosclerotic model in the last part of the 20th Century and the first years of the 21st. Atherosclerosis was then considered as a blood vessel inflammatory disease, with a complex immune interaction with blood cells and cytokines [2]. This

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hypothesis was clinically demonstrated in some recent key clinical trials. In particular, the CANTOS trial demonstrated a reduction in cardiovascular mortality in patients who received IL-1 antibody [5]. And in addition, colchicine, a classic immunomodulatory drug, also demonstrated some molecular and clinical promising results in coronary artery disease patients, in particular in terms of reduction of inflammatory markers [6]. Thus, the theoretical framework of acquired mutations as the trigger and immunity and inflammation as the mechanism responsible for the amplification of the disease was established. A significant amount of experimental and clinical evidence has increased our understanding regarding this important cross-talk.

1.2. The First Challenge: From Endothelial Progenitor Cells to a Bone Marrow Failure

Nevertheless, the idea that blood and bone marrow had a deeper protagonist in atherogenesis beyond being a mere reactive spectator became to gain momentum. The seminal work by Asahara et al. [7], demonstrated that bone marrow progenitors originated from the bone marrow could engraft and repair the endothelium in adults. With this precedent, a huge work was performed on the research of these progenitors, with a direct clinical application for the diagnosis (possible reduced levels as a putative risk factor) and treatment (usage of these cells as a therapy) [8], Schmidt-Lucke. However, both approached found complexities. Considering cardiovascular disease as a mere quantitative deficit in endothelial progenitor cells and considering the best treatment as the one that could increased them soon became too simplistic.

For the diagnosis, levels of entothelial progenitor cells could increase as a response to ischemia, being low in healthy patients, high in ischemic patients and suboptimal in atherosclerotic patients. This was the case, for example, in cardiac surgery patients, having valvular patients increased levels of CD34+/CD144+ progenitors compared to healthy controls, whereas coronary artery bypass grafting patients yielded intermediate numbers, what suggested suboptimal mobilization [9]. This impaired mobilization could also explain the link between a decreased number of these cells during exercise and subsequent cardiovascular risk [10].

Then, the question was why these endothelial progenitor cells were released from the bone marrow at a lower extent in patients with cardiovascular disease.

The concept that these cells could have an impaired mobilization was clearly demonstrated by Fadini et al., who showed a suboptimal CD34+ progenitors mobilization in diabetic patients, with morphological differences in the bone marrow by an acquired difference in the bone marrow niche [11]. Oncostatin M was a key mediator in the impaired mobilization of CD34+ cells by the stroma [11]. This coincides with the clinical observation that, for the clinical hemispheres procedures to treat hematological diseases (autotransplant or allogenic donors), patients with diabetes and obesity tend to be poor CD34+ mobilizers.

The question arises why these CD34+ are sequestered by the innate immune system and whether these CD34+ cells were normal or rather they already came from the bone marrow in a disease state.

1.3. Lessons from the Clinic: Myeloproliferative Disease Is a Human Inflammation Model for Both Atherosclerosis and Cancer

Myeloproliferative neoplasms (MPN) are a group of hematological diseases originated from the neoplastic proliferation of hematopoietic stem cells that have thrombosis as the major cause of morbimortality in the natural history [12]. Thus, MPN are considered as a human inflammation model of cancer cell development, a concept deeply revised by Hasselbalch [12]. In fact, MPN patients tend to have an accelerated atherosclerosis, which explains the high rate of cardiovascular disease beyond increased cell counts [13]. In fact, MPN are increadingly being considered as both bone marrow, blood and vascular diseases, and in some clinical cases the arterial or venous thrombosis can herald the diagnosis of the disease [12]. Myeloproliferative neoplasms are clonal neoplasms originated from the hematopoietic stem cell. In particular, driver mutations in the JAK/STAT pathway play a major role in the pathogenesis of this group of diseases [12]. Of note, myeloproliferation and JAK/STAT activation have been noted in atherosclerotic lesions, whereas cholesterol has been demonstrated to act as the fuel of myeloproliferation in atherosclerosis [14].

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1.4. On the Democratization of Genetic Analyses: JAK2V617F

Given that one of the most important genetic drivers in MPN to activate the JAK/STAT pathway is the mutation JAK2V617F, the question arises whether this mutation appears in the general population and whether it increases cardiovascular risk or not. Populations studies have shown that this hypothesis is correct, being this mutation present in the 4,4% of the general population, whereas it appears in 11,3% in patients with ischemic stroke in a large population study performed in Denmark [15].

This observation links with the clinical observation that JAK2V617F is the most pro-thrombotic mutation in MPN. In fact, it has been demonstrated to herald the diagnosis of MPN after the coming of an atypical trombosis (eg. Budd-Chiari syndrome) [16].

Moreover, JAK2V617F mutation is present in leukocytes, platelets endothelial cells, and increases the expression of cell adhesion molecules and tissue factor, which increase both thrombosis and vascular inflammation [17]. Thus, JAK2V617F mutation can be considered as a trigger for not only myeloproliferation but also for accelerated atherosclerosis.

1.5. When Clonal Hematopoiesis Took Central Stage

Thus, so far we have seen some pieces of a puzzle. 1) Atherosclerosis is a bone marrow disease that drives altered CD34+ from the bone marrow, being a bad seed on a good soil. 2) MPN are a model for atherosclerosis. 3) In some cases, atherosclerosis is itself a MPN with typical mutations such as JAK2V617F, which accelerates myeloproliferation, vascular inflammation and eventually thrombosis.

The puzzle was finally completed when technology made it possible. The generalization of genetic panels allowed the study of multiple mutations in multi-genes platform panles at the same time with a strong genetic precision. Myeloid panels, designed for the diagnosis of myeloid hematopoitic diseases (MPN, acute myeloid leukemia and myelodysplasia), were tested in general populations. In a large study published in 2014, the presence of a somatic mutation was associated with an increase in the risk of hematologic cancer (hazard ratio, 11.1; 95% confidence interval [CI], 3.9 to 32.6), an increase in all-cause mortality (hazard ratio, 1.4; 95% CI, 1.1 to 1.8), and increases in the risks of incident coronary heart disease (hazard ratio, 2.0; 95% CI, 1.2 to 3.4) and ischemic stroke (hazard ratio, 2.6; 95% CI, 1.4 to 4.8) [18]. Moreover, in a more recent specifically designed to address the link between this condition and cardiovascular disease, mutations in the myeloid were associated to a risk of coronary heart disease that was 1.9 times as great as in noncarriers (95% confidence interval [CI], 1.4 to 2.7)., being JAK2V617F the mutation with a strongest link to future events, with a hazard ratio of 12 (95% confidence interval [CI], 3.8 to 38). [19]. Therefore, in the general population, clonal hematopoiesis can herald the coming of atherosclerosis in a similar manner to what happen in MPN patients, which opens new venues for early diagnosis and putative pre-emptive treatments in the future [19]. In particular, JAK2V717F is a target of particular importance for patients of MPN [17] and subjects with mutations who are at the highest risk to develop cardiovascular disease among all CHIP variants [19]. Therefore, MPN is a model of myeloproliferation, which eventually drives atherogenesis. This vision summarizes the findings so far from inflammation to clonal hematopoiesis of indeterminate potential. Therefore, to say clearly, you have the age of your heart. Your heart has the age of your arteries. Your arteries have the age of your endothelium. Your endothelium has the age of your blood and, not a surprise for hematologists, your blood has the age of your bone marrow.

2. Clonal Hematopoiesis and Its Clinical Application

2.1. General Concepts

The seminal work by Jaiswal at al. [18], put the emphasis on the importance of the concept of clonal hematopoiesis as a herald for the coming of future hematopoietic myeloid neoplasms and cardiovascular disease. Of note, this clonal hematopoiesis can be detected in peripheral blood, a tissue with universal access in all clinical circumstances and from both diseased patients and healthy

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subjects. The current concept of clonal hematopoiesis of indeterminate potential (CHIP) implies a variant allele frequency (VAF) of at least 2%, which implies that 4% of the blood nucleated cells do possess this mutation [20]. We have to note that the threshold of 2% is arbitrary. A low VAF (0,2%) can be detected with the most sensitive techniques, and it is almost universal in subjects older than 50 years old [21], whereas a threshold of VAF 10% is more related to future diseases, both myeloid cancers and cardiovascular disease, however this increased specificity may be gained at the cost of a lower sensitivity [19].

From the CHIP mutated genes, the most frequent are in the very first place of frequency DNMT3A (which takes account of approximately half of cases of CHIP) and second TET2 [18]. Both genes are related to DNA methylation and thus regulate hematopoietic stem cell renewal and subsequent first steps of genetic instability [22,23]. Thus, they could be understood as the first steps in the acquisition of genetic instability and eventual clonal evolution.

2.2. CHIP and Myeloid Neoplasms

In general, the risk to develop a myelodysplasic neoplasm (MDS) after CHIP is up to 1% per year [24]. It is possible to separate the causes that have the greatest probability to develop MDS by applying the rule that 2 or more mutations, any mutation more than 10% VAF or high-risk mutations for the development of secondary neoplasms, such as IDH1, IDH2, genes for the splicing (SRF2F, SF3B1), or genes related to metabolic pathways (JAK2V617). These high risk patients may benefit from an early bone marrow aspirate and biopsy to determine the disease and establish prognosis and future therapeutic strategies. Nevertheless, so far there is nothing we can do to stop or retard the clonal progression from CHIP to hematologic malignancy [18].

The current clinical approach for patients with CHIP and suspected myeloid neoplasm is to actively reach it by bone marrow biopsy in care a maintained cytopenia is present, as well as the presence of two or more CHIP mutations, any mutation with 10% VAF or more, or mutation in high-risk genes for cancer progression such as TP53, IDH1/2 or splicing-related ones [24], Table 1.

However, this is only a general management rule that does not take into account the concept of cytopenia. Clonal cytopenia of undeterminate significance (CCUS) is a condition defined by a cytopenia driven by a clonal hemapotopoiesis in the absence of clonal myeloid neoplasms. The introduction of the concept of cytopenia strengthens the necessity to deepen into the knowledge of the case. It is expected that, in addition to cytopenia, other markers of the complete blood count related to dysplasia (such as high mean corpuscular volume and high red cell distribution width), will be included in future algorithms for the management of these patients. Massive data analysis such as machine learning techniques could help to sharpen and individualize the algorithms for an early diagnosis by combining cytopenia, other blood counts assessments, and clonal hematopoiesis. Then, a personalized risk profile could be identified depending on these factors.

Table 1. Clinical algorithm for the management of patients with CHIP and high risk of development of myeloid malignancy [24].

Two or more CHIP mutations (any ≥2% VAF)	High risk of malignant hemopathy Consider bone marrow biopsy
Any mutation ≥10% VAF	
Mutations in TP53, IDHa/2, splicing genes	

It is tempting to speculate whether a pre-emptive treatment may prevent the progression from CHIP to MDS by using medications such as hypomethylating agents. However, clinical trials in this filed need a very long time to show any putative positive result, which is an important challenge. It could be more practical to focus on the most important pathways and test in vitro inhibitors of CHIP progression. In addition, focus on surrogate markers (eg. clonal evolution measured by VAF amount) may help to designed clinical trials with a shorter follow-up as a proof of concept. In the last years, many pathways and compounds are opening new venues of this line of research in experimental models, such as IDH1/2 mutant–derived 2-hydroxyglutarate and derived TET inhibitors [25]. Other

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interesting pathways that could be modulated to decrease CHIP progression are the interleukin 1 receptor-associated kinase (IRAK) [25] and the inflammasome [26].

If it could be eventually possible to avoid the progression of myelodsyplasia by drugs or metabolic products, the scenario would really resemble the cases of vitamin B12 or folate deficiency, where cytopenia and morphological dysplasia can be reverted by a simple treatment. However, the clinical development journey of the compounds designed to act on clonal hematopoiesis does not seem such an easy battle. First of all, these or other novel putative compounds for the treatment of CHIP would need a phase I study on healthy subjects with CHIP and test whether it is possible to stop or even reverse the process of the clonal evolution in a reasonable amount of time. For the phase II, a cross-sectional design -could be of interest, given that it could be proven in healthy controls as well as patients with MDS at different subtypes and stages of the disease to test the same hypothesis, as well as to relate these genomic changes with hard clinical endopints (mortality, blasts, morphology, evolution to acute leukemia). And eventually, after we have the necessary knowledge about the potential of these compounds in subjects with CHIP, as well as in patients with MDS, a long-term longitudinal design would be expected to the yield the maximal performance.

With a similar approach, CHIP can also be used as a herald for the coming of a future acute myeloid leukemia. Several studies, by using deep-sequencing techniques, have demonstrated the possibility to predict the coming of an acute leukemia by several years, and the pre-leukemic samples were different from controls [27]. Yet again, so far there is nothing we can do to avoid this fate. However, this situation may change in the future by using similar approaches than the ones used in MDS. In addition, strong and reliable predictive models for AML have a direct clinical application: the avoidance of disease cases with hyperleukocytosis and clinical instability, which are linked to a higher mortality, in particular in the induction phase. Similar to what happens in multiple myeloma, where patients who come from clonal gammopathy of unknown significance seem to have a better evolution that de novo cases, it is expected that prediction will help to design the best treatment and fit the best supportive care, as well to avoid the worst situations at the induction phase.

2.3. CHIP and Cardiovascular Disease

The conceptual journey form cardiovascular disease to CHIP took time and effort. However, the back travel is going very fast, since the assessment of CHIP is becoming a new independent cardiovascular risk factor, in particular when JAK2 mutation is present, or where there is a VAF higher than 10% [18,19], Table 2.

Table 2. Clinical algorithm for the management of patients with CHIP and high risk of cardiovascular disease [18,19].

Mutation in JAK2 (any ≥2% VAF)	Derivation to cardiovascular risk specialist
Any mutation ≥10% VAF	Cardiovascular image?

As reviewed above, in experimental models, JAK2 activates JAK/STAT, which is directly atherogenic [12]. Moreover, it has been also demonstrated that TET2 and DNMT3A mutations directly trigger atherosclerosis by involving IL-1 and IL-6 [22,23]. Nevertheless, there seem to be some differences in the way these two frequent CHIP mutations drive atherogenesis. TET 2 inactivation increases IL-1beta, IL-6 and CCL5, whereas DNMT3A loss of function increases the expression of CXCL1, CXCL2, IL-6 and CCL5 [28].

Contrary to what happens in cancer, here the clinical management application seems immediate: the possibility to perform non-invasive cardiovascular imaging and thus predict the coming of future cardiovascular events, which can be avoided, at least in part, by classic and novel cardiovascular drugs and acting on risk factors. Moreover, in the future it could be possible to design or use targeted therapies in a similar manner to what is currently being tested in laboratoy models of myeloid neoplasms. In a pre-clinical experimental animal model, a blockade of IL-6 reversed TET-2-mediated atherosclerosis in mice [29]. From the clinical point of view, a sub-analysis of the CANTOS trial, patients with CHIP due to somatic mutations in TET2 also had reduced risk for major adverse

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cardiovascular events while taking the IL-1 inhibitor canakinumab (hazard ratio, 0.38 [95% CI, 0.15-0.96]) [30].

However, as discussed above, from the mechanistic point of view, one of the most attractive candidates in the JAK/STAT pathway, given that it drives both myeloproliferation and atherosclerosis in MPN patients [17,19]. The possibility to be inhibited in a specific manner in the mutation held in the pseudo-kinase domain makes it the ideal target. Specific inhibitors for JAK2V617F mutations that are currently in pre-clinical development for MPN [31] and well-designed clinical trials may prove the possibility of these targets not only to stop the progression of MPN, but also to potentially reverse atherosclerosis in MPN patients and in subjects with JAK2V617F CHIP, who are the highest risk to develop atherosclerosis among all CHIP carriers [19]. In addition to the evident clinical achievement, the regression of atherosclerosis by using a JAK2V617F inhibitor would close the circle between this driver mutation, myeloproliferation and atherosclerosis. The logical clinical development of these compounds, however, will probably begin with patients with MPN with proven cardiovascular disease, where atherosclerosis regression by non-invasive image techniques may be the first step towards other studies with hard cardiovascular endpoints to be determined. If these endpoints are demonstrated in MPN patients, the next logical step would be to extend the benefits to subjects with JAK2V617F clonal hematopoiesis and absence of overt MPN diagnosis.

2.4. CHIP, Solid Tumors and Secondary Neoplasms

There are some specific clonal markers for the coming of therapy-related myeloid secondary malignancies after chemotherapy and/or radiotherapy such as PPMD1 [32] and TP53 [33].

For patients who underwent autologous hematopoietic stem cell transplantation (a procedure that implies high-dose chemotherapy as conditioning regime), 29.9%) had CHIP at the time beforehand, which was associated with an increased rate of therapy-related myeloid neoplasm (10-year cumulative incidence, 14.1% v 4.3%; P = 0.002) [34]. The prediction of the patients that may develop treatment-related myeloid secondary malignancies may help to tailor a personalized approach in treatment design in selected cases by avoiding the most intensive treatment in these selected cases and use other treatments such as radiotherapy instead. Well-designed trials to address this issue are eagerly awaited. The good news it that, given that we already have CHIP, with is a reproducible surrogate marker of eventual coming of second cancer, it will be much easier to design these studies, given that it will not be necessary to wait for years or decades until the re is a difference in the rate of secondary neoplasm. Given the emerging importance of secondary neoplasms in all cancer treatment designs, it is expected that the algorithms of the future will incorporate clonal hematopoiesis as important factors to be taken into account, in a similar manner than co-morbidity scales are taken into account to design the best oncological treatment for every single patient in a tailored approach.

3. Conclusion

The concept of CHIP has changed our vision of both atherosclerosis and cancer, the two leading causes of mortality. It is expected that the wider use of genetic panes will expand the utilities for both early diagnosis and pre-emptive treatments.

Early diagnosis has an immediate clinical application in atherosclerosis. Given that CHIP is increasingly being recognized as a new independent cardiovascular risk factor, it may be possibly included in coming cardiovascular risk scores, that may imply derivation to specialized management and possibly the help of non-invasive cardiovascular image with the avoidance of cardiovascular deaths. In particular, cancer survivors have an unexpected increased cardiovascular risk, may benefit this approach [35]. Moreover, treatments applied to the CHIP itself may reverse atherosclerotic lesions as observed in experimental animal models and some clinical observations. This approach can, in the next years and decades, became a clinical game changer in cardiovascular disease.

For myeloid neoplasms, the possibility of an early diagnosis and proper management of the disease may lead to the avoidance of the most advanced cases, the ones who have the poorest clinical

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outcome. A proper prediction and management may lead to patients where diagnosis and treatment of overt hematopoietic neoplasms will take place at the right clinical moment, in a similar manner to what happens in other hematologic malignancies that have been preceded by the asymptomatic clonal state (eg. monoclonal gammopathy of clonal lymphocytosis of indeterminate potential). However, the most attractive concept: the possibility to use pre-emptive treatments to stop or even reverse the evolution for CHIP to myeloid malignancy, an approach that may prevent the coming of this group of potential devastating diseases. A great laboratory research effort should be followed by well-designed clinical trials, where CHIP could be considered as the primary endpoint since it role as a surrogate marker of future myeloid neoplasms is now clear and we have common and reproducible techniques and definitions. A long-term collaborative effort is essential for the success of this journey ind it is expected to take a long, but passionate, journey.

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