



**Universiteit  
Leiden**  
The Netherlands

## **Predictive factors for outcome of rheumatoid arthritis**

Linden, M.P.M. van der

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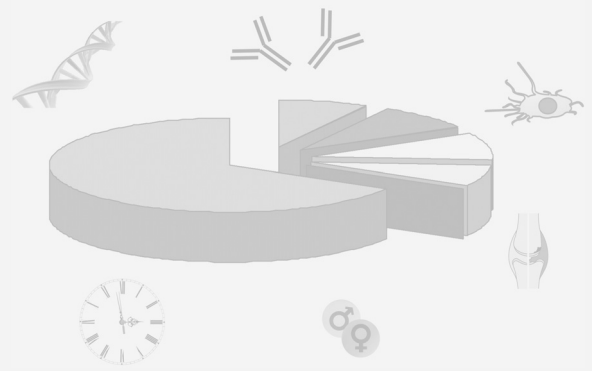
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# CHAPTER 15



## Summary and Discussion

## SUMMARY AND DISCUSSION

RA is a chronic and progressive autoimmune disease affecting approximately 1% of the population worldwide, and has a large risk for causing disability of patients and consequently high costs in health care if left un- or improperly treated. To prevent this, patients with RA need to be identified as early as possible and treated adequately to prevent worse outcome. Early recognition, together with prediction of the disease outcome at the individual patient level would allow to achieve personalized medicine. The main scope of this thesis was to identify and evaluate the quality of risk factors for their usefulness in predicting disease course and outcome of RA. To this end, characteristics and disease outcome of RA patients included in the Leiden EAC were studied.

To treat patients with RA adequately and swiftly, the first requirement is a tool to correctly identify patients with early RA. The benefit of early detection and treatment has been recognized many years ago. Although the 1987 classification criteria for RA incorporated a minimally required symptom duration of 6 weeks, the majority of the items of these criteria relate to long standing RA.<sup>1</sup> Although the 1987 criteria have been used for many years and were considered as a huge improvement over the criteria for RA that were formulated in the 1950s,<sup>2</sup> still, the 1987 criteria perform rather poorly in defining RA in a early disease phase.<sup>3,4</sup> The advancing knowledge of the disease course of RA and the need to perform trials in early RA have led to the development of a new set of criteria. These 2010 ACR/EULAR criteria, which now include acute phase reactants and an updated item serology, were devised with the intention to classify patients in an earlier stage of the disease.<sup>5</sup>

Although in the process of deriving the new criteria multiple datasets have been used,<sup>6</sup> their performance in various individual datasets will be the focus of attention in the coming years. Our study (**chapter 13**) indicates that the 2010 criteria, in comparison to the 1987 criteria, classify more patients as having RA. Most importantly, these patients are indeed classified in an earlier stage of the disease. The observation however that an increased number of patients is classified with RA, may indicate a certain degree of false positive misclassification, an expectation that we seem to confirm with our findings. We observed that, depending on the population, 9 or 18% fulfilled other diagnoses during their first year of the disease. Psoriatic arthritis was the most frequent cause of “misclassification”. Although the user manual of the 2010 criteria clearly states that these new criteria should only be applied to a set of patients that cannot be classified with another rheumatologic diagnosis, it would be most interesting to be able to use these criteria for every new arthritis patient that visits a rheumatologist. Another issue is that the presence of erosions is ‘*prima facie*’ evidence for the classification of RA within the new criteria, meaning that the other criteria do not have to be fulfilled.<sup>5</sup> Although not a focus of attention in this thesis, the exact definition of an erosion typically for RA should be defined. This has to some extent already been the subject of discussion.<sup>7</sup> A third issue that has not been settled yet, is how an increased RF

level can be defined. As shown in **chapter 4**, the variance in IgM-RF level obtained with different methods is considerable. When evaluating three different outcome measures (MTX initiation during the first year, initiation of any DMARD during the first year and arthritis persistency over 5 years), the new criteria showed a better sensitivity but a lower specificity for the outcome of RA compared to the 1987 criteria. However, since this is the first study published on this topic, replication in other cohorts is needed. Nonetheless, the 2010 criteria could well be regarded as a first step towards earlier recognition of patients at risk and the development of a more accurate set of criteria.

The importance of an earlier detection of RA has been clearly shown by various studies that observed beneficial effects of treating patients as soon as possible. The increase in evidence during the last decade for the existence of a so-called “window of opportunity” of 3 months in which RA patients are most sensitive to treatment,<sup>8-10</sup> is a good example of the growing awareness of the importance to treat early. The effect of treatment within this 3 month period on the effectiveness of response to therapy has been established with significant associations in terms of better outcomes of RA.<sup>11,12</sup> In particular, Finckh et al<sup>13</sup> indicate that early treatment (<3 months) would not only limit the amount of joint damage that can accumulate prior to treatment but in addition it can also slow down the rate of progression afterwards. Although the period of 12 weeks may be somewhat arbitrary, evaluating the effect of early assessment (<12 weeks) in the present study yielded significant support for the importance to treat early (<12 weeks) in terms of long-term outcome of the rate of joint destruction over 6 years and the achievement of sustained DMARD-free remission (**chapter 9**), thereby strengthening prior investigations. Studying the effect of delay on the rate of joint destruction in ACPA subsets showed that the observed association remains present in the ACPA-negative group of patients. Notably, although we did not unequivocally establish a similar result in the ACPA-positive patients, a similar tendency however was observed (**chapter 9**). Although it needs to be considered that this is an observational study and not a randomized trial so it may be that these patients had an innate difference in outcome, we have not been able to identify such differences. Extension of the radiographic data in **chapter 10**, revealed that the effect of delayed assessment on the rate of joint destruction was statistically significant as well in ACPA-positive RA. This indicates that the suggested “window of opportunity” might apply to both ACPA-negative and -positive RA, but that we lack sufficient statistical power (**chapter 9**). We also observed that among all early arthritis patients ACPA-positive RA patients had the longest delay, and at least 77% of them were assessed after 12 weeks of symptoms. We evaluated whether the “window of opportunity” was explained by different characteristics of the ACPA response (**chapter 10**) and observed that patients that were assessed within or after 12 weeks of symptoms had comparable numbers of isotypes or recognized peptides. The observed lack of an association between the broadness of the ACPA-response and the groups with <12 weeks and ≥12 weeks of delay, might indicate that maturation of the autoantibody response occurs even earlier. This notion would be in line with the observation that autoantibodies, among which ACPA, are already present in the serum of future RA patients in the preclinical phase

several years before the onset of symptoms.<sup>14</sup> The observations that the autoantibody response appears to be initiated before symptom onset, and that levels of acute phase reactants and markers reflecting alteration of bone metabolism are simultaneously elevated as well,<sup>15-17</sup> could lead to the hypothesis that the “window of opportunity” does not lie in the first 12 weeks after initiation of the first symptoms, but is actually located before the clinical onset of disease.

Having established a solid foundation for the need to treat early it is important to raise the question where the observed delay comes from. Since the EAC is organized such that referred patients can visit the rheumatologist quickly (~1-2 weeks), the duration of the delay time, defined as the time between the onset of symptoms and the first visit at a rheumatologist, can in this case be roughly divided into two parts: it could lie either at the patients' end, by reluctance to seek medical care, or at the end of their general practitioner (GP), by referring a patient in a later stage than would be preferred. Although this subject has been studied before and to some extent is subject to geographical variation and depending on the organisation of health care in a particular nation, our study shows that the main contributor to delay in assessment in the Netherlands is the GP delay (chapter 9). An important element in this study was to identify a profile that characterises the patients that have the longest delay. Notably, strikingly similar to the classical image of RA,<sup>18</sup> among all early arthritis patients the current observations were that older age, female sex, gradual symptom onset, involvement of the small joints, lower levels of C-reactive protein, and the presence of autoantibodies were associated with longer total delay. Thus although confirming the general idea about RA, this indicates the need for active and increased awareness to decrease delay time in the future.

The second step in achieving personalized medicine, after early recognition of RA, is to obtain the ability to predict the long-term outcome of RA. To identify risk factors, to this end, two main outcomes used to identify new risk factors for the severity of RA were studied: the rate of joint destruction and the achievement of DMARD-free remission. In this thesis we analysed longitudinal data using a powerful statistical approach (**chapter 2**) that takes maximal advantage of the presence of serial radiographs observed in studies. The ultimate purpose of identifying risk factors is to put together a risk profile for the individual patient, that can lead to the composition of an adequate prediction rule.<sup>19,20</sup>

Although some studies on genetic risk factors for the severity of RA have been performed, fairly little is known about this subject. Most importantly, most observations done thus far, are single studies which have not been replicated. Years of experience however indicate that the replication is in fact needed to prevent false positive findings.<sup>21</sup> In addition, false negative findings should be avoided. Since the effect sizes observed in genetics association studies are in general small, effects may be lost in case of too much noise.

In **chapter 7**, we show that the minor alleles in two loci, rs675520 and rs9376293, located on chromosome 6q23 in a region close to the gene encoding for *TNFAIP3*, associate with a higher

rate of joint destruction within ACPA-positive RA. Since this was the first study to show an association between SNPs located in the *TNFAIP3* region, replication of our findings for the effect on the severity of RA however is needed to confirm the validity of our findings. Nonetheless, when looking at the role of *TNFAIP3* region in RA susceptibility, it can be suggested that the region has similar influences not only on the risk for development but also for a worse outcome of RA, and that the effect in both cases is confined to the ACPA-positive subset of RA patients. For two previously identified susceptibility risk loci in this locus, rs6920220 and rs10499194,<sup>22,23</sup> no effect on the rate of joint destruction was observed however.

A similar observation was done for *PTPN22*, a genetic region previously identified as a risk factor for susceptibility in ACPA-positive RA. As described in **chapter 9**, no association was found between the polymorphism and the rate of joint destruction. Notably, this observation was done in two independent cohorts and thereby provides further indication that *PTPN22* does not affect the development of damage to the joints of RA patients, but may primarily have a role in predisposing to the emergence of ACPA.<sup>24</sup> Other risk factors that also showed similar discrepancies between the risk for susceptibility and severity are *KIF5A/PIP4K2C*, *CDK6*, *CCL21*, *PRKCQ*, and *MMEL1/TNFRSF14* (**chapter 8**). Taken together, these observations might indicate that a risk factor for susceptibility is not necessarily always a risk factor for severity of joint damage in RA as well.

Inconsistent effects for RA susceptibility and severity were also found in **chapter 8**, where we investigated the effect of a SNP in the *CD40* gene (rs4810485) on the rate of joint destruction in ACPA-positive patients. Our results show that, in two independent cohorts, ACPA-positive RA patients homozygous for the minor T allele were characterized by significantly higher rates of joint destruction. However, counterintuitive to what one would expect, associating with a less severe course of RA, the common (G) allele conferred risk to develop RA.<sup>25</sup> As pointed out in this chapter, the disease-associated (common) allele marks a haplotype of *CD40* that contains a polymorphism in the upstream Kozak sequence that results in increased surface expression on B cells.<sup>26</sup> In addition, it has been reported that CD40 expression is increased on synoviocytes in RA, and triggering of CD40 in synovial fibroblasts is associated with the production of proinflammatory cytokines and osteoclastogenesis.<sup>27,28</sup> The likeliness that the biologic pathways underlying susceptibility and severity are distinct, in this case with respect to the triggering of CD40, would provide an explanation for the observed discrepancy and in theory could, at least partially, provide an alternative explanation for the discrepancies that are observed for other polymorphisms.

A special role in the pathogenesis of RA is fulfilled by the presence of autoantibodies. These autoantibodies characterize the derailment of the autoimmune system, intended to protect the human body from allogenic threats, by showing a cross-reaction with autoantigens and a subsequent activation of immune responses. Presence of these autoantibodies has firmly been established as associating factors with increased development and severity of RA.<sup>29</sup>

Various tests (IgM-RF, anti-CCP2, anti-CCP3 and anti-MCV) have been manufactured and all have shown to be useful in the process of detecting autoantibodies present during the process of RA. These tests have all individually been reported to have adequate characteristics in terms of performance in terms of sensitivity and specificity, but a head-to-head comparison has never been performed. Although overall anti-CCP2 tended to have the best performance, we find that, evaluating all these tests for a positive or negative test result, no large differences were observed between either test (**chapter 3**) for both the development of RA as well as the rate of joint destruction and the achievement of DMARD-free remission. These results are not surprising since the proportion of patients with presence of more than 1 autoantibody was over 71%, indicating a large coexistence of these autoantibodies. Presence of more than one autoantibody however was associated with worse outcomes. In addition to the presence of ACPA, IgM-RF did not have a significant additive contribution. This also suggests that the predictive value of ACPA is larger than that of IgM-RF.

In the updated item serology of the new 2010 criteria now also the use of ACPA was included in addition to RF.<sup>5</sup> Notably, in addition to the mere presence, the levels of these autoantibodies were given weight as well in the process of classifying a patient with RA. Although higher levels of autoantibodies have been shown to display a higher specificity and associate with an increased development and a higher severity of RA than autoantibody positivity,<sup>30-32</sup> we show that the presence of ACPA also performs better than raising the used cutoff for RF-positivity (**chapter 4**) in addition to the presence of RF (**chapter 3**). In **chapter 4**, the presence of ACPA performed better for predicting the development as well as the outcome of RA. Moreover, performing a RF test in ACPA-negative patients did not prove to be valuable, while determining ACPA in RF negative patients did contribute. Therefore, we propose to omit the use of RF from the 2010 criteria.

IgM-RF is frequently observed in other inflammatory diseases<sup>33,34</sup> and is sometimes present in healthy older persons,<sup>35</sup> suggesting that RF can be a consequence of nonspecific immune activation. In contrast to IgM-RF, antibodies to anti-citrullinated proteins are highly specific for RA.<sup>36</sup> It has been suggested that IgM-RF production is a consequence of the rheumatoid inflammation whereas ACPA may have pathophysiological properties. Moreover, it is presumed that the association of RF with the presence of RA is primarily explained by its interaction with ACPAs.<sup>37</sup> However, formal proofs that ACPA are causal for RA are lacking.

It has been hypothesized that two different subsets of RA can be characterized by the presence or absence of ACPA.<sup>38,39</sup> This hypothesis is supported by the observed differences in risk profiles for both genetic factors (references,<sup>25,40,41</sup> **chapter 7 and 8**), environmental factors<sup>42</sup> and their interactions, as well as a different reaction to methotrexate treatment in both subsets.<sup>43</sup> Fully understanding the differences between ACPA-positive and ACPA-negative RA as separate entities, especially the underlying molecular pathophysiology, might elucidate the etiology of RA.

Inflammation of the synovium in a rheumatoid joint is a key process in RA, and the intensity and duration of such an inflammation is largely depending on the interplay between different

cell types of the immune system that are localized either in the joints, like fibroblast-like synoviocytes, and cells that roam the human body and are attracted to sites of inflammation, like dendritic cells, macrophages and B- and T-lymphocytes.<sup>44,45</sup>

In chapter 5, we show that the CXCL13, a cytokine that selectively attracts B cells<sup>46</sup>, significantly associates with the amount of joint damage in terms of erosiveness and the total Sharp/van der Heijde score. Higher serum CXCL13 levels corresponded with higher rates of joint destruction. The effect was independent of the inflammatory marker CRP, with which the level of CXCL13 on itself is also correlated. Subsequent treatment with anti-TNF $\alpha$  therapy has been reported to significantly reduce CXCL13 serum levels.<sup>47,48</sup> Evidence for joint localization of CXCL13 has been found, both by the detection of mRNA in inflamed synovial tissue<sup>49</sup> as well as the presence of ectopic lymphoid follicles expressing CXCL13 in the synovium of chronic RA patients.<sup>50</sup> Importantly, formation of these ectopic lymphoid follicles has been implicated in initiating and maintaining the inflammatory response in RA.<sup>51</sup> In addition, they have been suggested to associate with increased disease severity and accelerated breakdown of self-tolerance,<sup>52</sup> have been attributed a role in the priming and antigen presentation, and possibly contribute to initiating and maintaining the production of ACPAs, although this latter has not been unequivocally established.<sup>53</sup> The observation that CXCL13 expression takes part in the same chain of events leading to the formation of ACPA, together with the data establishing ACPA as one of the strongest predictors for joint damage, could explain our observation that CXCL13 only shows an association in ACPA-negative RA and that the association is lost in ACPA-positive disease. Notably, high levels of CXCR5 (the CXCL13 receptor) were also found on human osteoblasts and activation by its ligand CXCL13 induced the release of extracellular matrix degrading enzymes. As such, CXCL13 may play a direct role in the process of bone remodeling as well.<sup>54</sup>

The involvement of *TNFAIP3* and *CD40*, genetic regions associated with the rate of joint destruction in RA (**chapters 7 and 8**) as well as susceptibility to RA, together with recent discoveries of other genetic associations -for RA susceptibility- with several genes relevant to this pathway, *TRAF1* and *REL*, especially in autoantibody-positive RA, might point to a central important role of the CD40/NF- $\kappa$ B signaling pathway.<sup>55</sup> As such, to increase understanding of the pathophysiology underlying RA, identification of the cell types that mainly drive this pathway would be of great interest and would propose new interesting targets for interrupting the disease process in RA.

The observed expression of CD40 on the surface of multiple immune cells, including the B-cells, might implicate that CD40 has a broader role in autoimmune regulation in general.<sup>56</sup> Notably, the risk genotype of *CD40* that associates with RA susceptibility but has a protective effect for RA severity (**chapter 8**), has been observed to cause enhanced expression of CD40 on B-cells in Graves' disease.<sup>26</sup> Interestingly, in RA, interaction of CD40 with its ligand, CD40L (CD154), potentially leads to various immune reactions. These include B-cell proliferation through regulation of CDK6 expression, selective attraction of B-cells by regulating CXCL13, germinal center formation, differentiation of B-cells into plasma cells that secrete large titers of



high-affinity antibodies, immunoglobulin class switching, memory B-cell development,<sup>57-61</sup> and affecting osteoclastogenesis by NF- $\kappa$ B/CD40-mediated bone destruction. The sustained presence of the IgM isoform of anti-CCP during the ACPA response that is observed early in the course of ACPA-positive RA, is indicative for ongoing recruitment of new B cells.<sup>62</sup>

Altogether, these findings are supportive for the notion that especially the recruitment and organization of B-cells in the synovium play a critical role in the persistency of arthritis in ACPA-positive RA. This would support the hypothesis of ACPA-positive RA being a B-cell driven disease that was first postulated more than a decade ago.<sup>63</sup> Indeed, modern therapies with B-lymphocyte-depleting agents have shown to be useful in treating ACPA-positive RA.<sup>64,65</sup>

Inflammation is the hallmark of RA and is regarded as the catalyzer leading to disturbances in bone homeostasis by influencing the balance between bone formation by osteoblasts and bone degradation by osteoclasts. This disbalance generally leads to erosions of the joints. The reciprocal processes lead to the occurrence of repair at these sites. Although the concept of repair is still less well accepted, the results from our effort to characterize the subphenotype of RA patients with repair (**chapter 11**), support the notion that repair does exist. In 7.2% of RA patients we observed radiological repair in one or more joints of the same patient. In addition, our results show that despite the absence of aggressive or biological anti-rheumatic treatment, repair occurs in part of the general RA population. Notably, the most frequent occurrence of repair was in the patients who had the highest degree of radiological damage. As mentioned, one of the explanations could be that, to detect this phenomenon, a relatively high degree of eroded bone lesions has to be present (**chapter 11**). This coincides with the observation that in general, the patients with repair simultaneously showed an overall progression in total erosion and Sharp/van der Heijde scores (reference 66 and **chapter 11**).

The observations done on erosions and repair support the idea that not only the occurrence of erosions but also the repair of joint erosions is based rather on the processes involved in local bone homeostasis than on a systemic reaction.<sup>66</sup> Indeed, in our study, repair occurred only in joints without joint swelling in the two preceding years, a finding similar to that done in the TEMPO trial.<sup>67</sup> These observations imply that inflammation drives bone damage, a mechanistic hypothesis that has generally been accepted.<sup>45</sup> Notably, it is recently also suggested that the presence of cartilage and bone breakdown components can induce inflammatory processes.<sup>68</sup> As such a “vicious circle” may be activated.

Thus, the classic paradigm is that inflammation leads to damage, and indeed in majority of cases inflammation goes hand-in-hand with joint destruction. Progressing insights however indicate that the relation between inflammation and joint damage might not be that straight forward and that inflammation and joint damage might have different causal pathways.<sup>68</sup> Evidence substantiating this notion is provided by the observation that, in reaction to treatment an uncoupling of synovitis and joint damage at the individual joint level was observed.<sup>69</sup>

Thorough evaluation of patients with a disconnection between joint inflammation and destruction may yield insight in the processes involved in the link between inflammation and bone destruction. To this end we selected extreme discordant phenotypes (**chapter 12**). We identified patients with persistent joint inflammation over time but after 5 years no erosions (4%), and patients with a very low inflammatory burden but highly progressive joint damage (11%). The high-inflammatory, non-erosive patients were less often autoantibody positive, showed more often an acute start of the disease, and had more inflamed joints. The low-inflammatory, high-erosive patients had a more chronic onset of complaints and were more autoantibody positive. In case of the latter group of patients, it cannot be ruled that subclinical inflammation is present which causes deterioration in of joint damage.<sup>70</sup> It would be very interesting to study whether genetic (rare) variants are associated with these subphenotypes of RA. Although a small number of patients are available, it has been shown in other diseases that studying these extreme discordant phenotypes may be the basis to valuable new findings.<sup>71,72</sup>

Summarizing the data presented in this thesis, in **chapter 14** we provide an overview of the implications of these data for the progression from UA to RA, the development of persistent disease, and the main scope of this thesis, the prediction of outcome in RA in terms of the rate of joint damage. In this study, the risk factors that were observed to associate with progression to RA and the development of persistent disease in UA patients showed to be largely the same, with a main focus on inflammatory markers and autoantibodies. When analyzing risk factors for the outcome of RA, as measured by the rate of joint destruction, the largest effect sizes were observed for the presence of autoantibodies. Other risk factors were inflammatory markers like SJC, CRP and ESR, BMI and, also described in **chapter 9**, the symptom duration (delay) at the first visit to a rheumatologist. Comparison of the identified risk factors for outcome of UA as well as RA again largely resulted in the same set of risk factors. Some risk factors, like a positive family history for RA, acuteness of disease onset, morning stiffness, BMI and several characteristics of joint swelling however were only risk factors for either one of the outcome measures. This observed discrepancy in risk factors for the outcome of UA and RA however is not surprising. We also observed discrepancies between several genetic factors and RA susceptibility and RA severity in **chapter 8** and **chapter 9**.

We show that when combining the individually associating risk factors (**chapter 14**), the overall explained variance for the severity of joint destruction is 32%. During previous attempts to derive prediction rules for the rate of joint destruction, still ~50% of the RA patients could not be adequately classified.<sup>73-75</sup> Although these studies did not include genetics, one can ask the question whether the use of genetics does live up to its expectation of being “the holy grail”. For predicting the development of RA from UA it has been observed, that a prediction model incorporating genetic factors did not show an increased performance compared to a prediction rule based on common clinical and serological risk factors alone.<sup>76</sup> Nonetheless, it has unequivocally

been shown that identification of genetic factors, especially in the light of the related concept of heritability, has a substantial influence on understanding the pathophysiology underlying the development of RA.<sup>77,78</sup> The general notion is that for genetics only the tip of the iceberg has been revealed thus far, indicating the need for identification of more and newer genetic risk factors. Moreover, the genetic risk factors identified might not only ultimately allow us to make enhanced prediction of RA development and outcome, but may also give us the opportunity to predict the response to therapy.<sup>79-83</sup>

Our observations might implicate that including genetic factors in predicting the rate of joint destruction can in fact contribute to an increased explanation for the rate of joint destruction, but that for optimal performance, since these factors are primarily identified in the ACPA-positive subset, it would be desirable for future studies to determine the explained variance in the ACPA-positive and ACPA-negative subgroups separately. Next to the inclusion of genetics, also the evaluation of other markers might increase the explained variance of 32% that we observed in **chapter 14**. For example, including CXCL13 (**chapter 5**) will increase the total variance explained, since at the individual level, this factor could explain ~7% of the rate of joint destruction.

In conclusion, huge advances in the understanding and treatment of RA have been made in the last few decades, resulting in dramatically improved perspectives of RA patients nowadays. Nonetheless, the ultimate goal of personalized medicine however has not yet been reached. Although limited in the complete picture of RA, the results described in this thesis may present one step further in the process of achieving individualized treatment decision making. Especially the identification of genetic and serological factors are useful for this purpose. Future studies, dedicated to the identification of more and newer risk factors might help in completing the picture.

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