cells in the blood and in tumors,[6] and it seems, therefore, tempting to speculate that the VEGF-A–producing wound monocytes share many similarities with TEMs. Finally, it needs to be determined whether the current findings translate to other types of (skin) damage, as model-specific differences for the implication of myeloid-derived VEGF-A have been reported.[7]

Interestingly, angiogenesis and immune suppression often go hand-in-hand, sharing similar molecular and cellular mediators.[8] Also in the context of wound healing, immune-suppressive cells would come in handy as safe-guards against inappropriate immune reactivity toward endogenous danger signals. Cells such as myeloid-derived suppressor cells (MDSCs), which include a monocytic (MO)–MDSC and a granulocytic polymorphonuclear (PMN)–MDSC fraction,[9] are best known for their ability to suppress the activation of various lymphoid and myeloid cell types, but are also potently pro–angiogenic. Although the authors argue that the early wound-infiltrating monocytes do not fully comply with MO-MDSC surface marker expression, the ultimate test would be to assess their T-cell suppressive capacity.

Later stages of the wound healing process are characterized by a dominance of large, granular $CCR2^{low}Ly6C^{low}$ myeloid cells, which is more consistent with the phenotype of mature macrophages. It is tempting to speculate that these macrophages are the progeny of the initial $CCR2^{+}Ly6C^{high}$ monocyte infiltrate, similar to what has been observed in growing tumors.[5] However, one cannot formally exclude the contribution of distinct monocyte subsets to the sequential skin wound healing phases, a mechanism that seems to be required for a normal healing of the injured myocardium.[10] In addition, it would be interesting to investigate whether this pool of later stage wound macrophages is the mere result of monocyte infiltration and differentiation, or whether local proliferation of these tissue macrophages[11] contributes as well. A remarkable observation is the switch in the VEGF-A origin from myeloid to epidermal in the course of wound healing. This illustrates the importance of correctly timed and probably also correctly localized VEGF-A production during tissue regeneration. As a matter of fact, the inappropriate production of VEGF-A by macrophages might lead to immature and dysfunctional vessels,[12] which would hamper the healing process.

The strictly timed interplay of cells and molecules in the course of wound healing as demonstrated in the report by Willenborg et al might not facilitate therapeutic intervention, for example, to correct healing deficiencies. The risk exists that any therapy (at the level of monocyte or VEGF availability, for example) might be given too early, too late, too much, or too little, and hence not yielding the desired effects. However, this report is setting the stage for further exploring the wound healing process and the identification of additional players involved.

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● ● ● **THROMBOSIS & HEMOSTASIS**

Comment on de Haan et al, page 656

Predicting individual risk of venous thrombosis

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de Haan et al have developed a risk score algorithm that is able to improve the individual prediction of venous thrombosis, taking into account information from multiple risk SNPs in addition to clinical data.[1]

Who is going to suffer a venous thrombosis or pulmonary embolism? Predicting the individual risk of any multifaceted disease is a very difficult task. The complexity arises from the occurrence of multiple risk factors (with some likely yet to be identified) and their interplay. For venous thromboembolic disease, a substantial portion of these risk factors are genetic.[2] There have been a few attempts to mathematically predict the individual risk of venous thromboembolism. One of them, by Eichinger et al, proposed a very simple risk score for recurrence in patients with previous spontaneous thrombosis,[3] and 2 others tried to predict the risk of a first thromboembolic event in asymptomatic subjects.[4,5] The risk score proposed by Hippisley-Cox and Coupland uses

14 variables, exclusively clinical and included comorbidities and concurrent medications.[4] In contrast, Heinemann et al also suggested a simple algorithm that included 2 genetic risk factors, that is, the Factor V Leiden and the Prothrombin G20210A mutation.[5]

In this issue of *Blood*, de Haan et al report an algorithm that incorporates a wider set of genetic risk factors discovered recently by genome-wide studies.[1] The predictive power of the previous models, mainly based on nongenetic risk factors, is significantly improved by including genomic information. To our knowledge, these results are the first that demonstrate that sophisticated probabilistic models, which combine clinical, environmental, and genetic variables, can be efficient and

useful predictors of the thromboembolic disease of an individual. One of the more striking results in the article by de Haan et al is the apparent ineffectiveness of a risk score composed of 31 SNPs compared with another composed of only 5 SNPs with the highest individual odds ratio for venous thrombosis (Factor V Leiden, PT G20210A, A1 blood group, 1 SNP in the *Fibrinogen γ* gene, 1 in the *Factor XI* gene).[1] Remarkably, these 5 genetic variants have a direct or indirect functional effect on hemostatic proteins, highlighting the role of coagulation on thrombosis risk. Considering that all 31 SNPs are independently associated with thrombotic risk, although sometimes small, a better predictive power should be expected when increasing the number of SNPs in the model. We hypothesize 2 explanations for this paradox. Perhaps some of the proposed SNPs are false-positive. More likely, new and more powerful mathematical tools to integrate all of this multiplicity of genetic information are needed. To date, only classical methods of logistic regression have been applied. These methods are unable to cope with the interaction among risk factors. It is also possible that other variables play an important role in thrombotic risk. In this sense, a limitation to the study by de Haan et al is the exclusive use of common genetic variants in the analysis of the risk. Using these variants, only a very small portion of the disease susceptibility is explained and this is one of the more frustrating findings of the giant genomic studies published to date.[6] One germane question is where the "missing heritability" underlying the complex and common diseases lies. Very probably, other rarer genetic variants, of the utmost importance at the individual level, must be included in the risk score of venous thromboembolic disease.

The clinical implications derived from the article by de Haan et al are clearly relevant because predictive medicine should be a high priority in prevalent diseases, such as cardiovascular disease and venous thromboembolic disease. The ability to objectively classify high-risk individuals will allow the best preventive strategies, both in asymptomatic individuals and in already affected patients (secondary prophylaxis). de Haan et al have placed one of the cornerstones of the edifice. To continue the construction of this building, given the enormous difficulties in addressing the complexity of the underlying disease, it is essential to use more and better items, such as the following:

• New, not yet known, genetic risk factors. It is necessary to continue searching.

• Incorporation of the rare genetic variants into the analysis of risk, with stronger effects in the carriers. Some of these rare variants are also affecting hemostatic factors, such as the A384S in the *SERPINC1* gene,[7] the R67X in *SERPIN10* gene,[8] or Ser219Gly in the *PROCR* gene.[9]

• In addition, all the protective factors should be incorporated to better estimate the actual individual risk of suffering the disease.

• In terms of clinical practice, new laboratory methods for DNA genotyping and performing simultaneous and fast multiple SNP testing (diagnostic chips) would be welcome.

• Use of other available biologic information, beyond DNA data, such as potential new markers based on plasma intermediate phenotypes such as thrombin generation, thromboelastometry, dynamics of the activated partial thromboplastin time, platelet volume, plasma levels of Factor VIII, and so on.

• More specific mathematical models able to integrate this multidimensionality of variables (clinical, genetic, genomic, and biologic) will be necessary to accurately estimate the individual risk.

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● ● ● **THROMBOSIS & HEMOSTASIS**

Comment on Pozzi et al, page 664

Liberating R169 promotes anticoagulant protein C

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In this issue of *Blood*, Pozzi et al demonstrate that removing an anionic cage promotes exposure of R169 thereby generating a protein C (PC) that is far more readily activated.[1]

In the future, it may be possible to deliver anticoagulant function at an injury site with less reliance on cofactor-assisted processes. Physiologically, zymogen PC is subjected to thrombin catalyzed hydrolysis in the presence of thrombomodulin. The resultant PC serves as a serine protease and inactivates Factors Va and VIIIa, 2 key members of the coagulation cascade.[2] In addition to its anticoagulant roles, activated PC also exhibits cytoprotective, anti-inflammatory, and profibrinolytic features.[2] The mutants described by Pozzi and colleagues may be used to design improved forms of therapeutic PC species whose activation properties can be controlled.

PC is part of the trypsin-like protease family. It is converted to a mature protease after cleavage at the R169-L170 (P$_1$-P$_1$') peptide bond by thrombin. A new N-terminus is created that reorients its position within the active site region and helps to establish the oxyanion hole and the primary substrate specificity pocket.[3] The membrane-bound protein thrombomodulin assists in inducing the conformational changes needed to promote hydrolysis and conversion of the PC into its activated form. Thrombomodulin