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DOCTORAL THESIS

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NOVELTIES IN MONITORING PEDIATRIC PATIENTS WITH THROMBOPHILIA

ABSTRACT OF THE DOCTORAL THESIS

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INTRODUCTION

Thrombophilia is a pathological condition based on changes in coagulation balance, which tilts the balance towards the tendency towards hypercoagulability. It occurs as a result of a hereditary and/or acquired predisposition and is a continuously increasing cause of morbidity in the pediatric population.

Thrombotic events in children have become an increasingly common problem, especially in pediatric hospitals. The prevalence of inherited thrombophilia in children who develop thrombosis varies substantially by population (Colucci G, 2020).

This paper looked at the usefulness and indications of profiling for thrombophilia in pediatric patients and highlighted the risk factors and triggers involved, as well as other important variables to consider when interpreting test results in children. The unique problems for genetic testing in children have been addressed in accordance with the literature.

The potential benefits of thrombophilia testing in children who have had a thrombotic event, as well as in those with a family history of thrombosis or thrombophilia, were discussed, with a focus on the current limitations of such an approach and the possibilities for improving investigation algorithms.

The theme chosen for this doctoral thesis was related to the current aspects for the monitoring of pediatric patients with thrombophilia, in the context of the increasing incidence of thrombotic events in the pediatric population. At present, there is no international consensus on the most effective methods for diagnosing a procoagulant status in children, for monitoring a pediatric patient who has gone through a thrombotic event, and for identifying the specific treatment or duration of anticoagulation or antiplatelet therapy ideal for preventing a recurrence.

Currently, in Romania, the protocols for diagnosis, treatment and follow-up of the pediatric patient with thrombophilia, as well as the treatment possibilities are extrapolated from the guidelines for the adult population. Also, we did not find studies carried out in the country, on batches of patients, serially monitored, both biohumorally and in terms of the use of anticoagulant or antiplatelet therapy. Also, not many analyses on this subject are available in the international literature in the context of the pediatric population with thrombophilia and thrombotic events. This context gives the originality of the present study.

Purpose, objectives, working hypothesis

The purpose of my study was to identify the most effective investigations and methods for monitoring pediatric patients diagnosed with thrombophilia, as a result of an acute thrombotic event, provoked or unprovoked, in order to choose the best treatment option so as to reduce the burden of long-term complications and the recurrence of thrombotic events.

The main objective of the thesis was to develop a personalized diagnosis, treatment and monitoring protocol in order to improve the quality of life of patients after an acute thrombotic episode that will consider adjusting the doses and periods of treatment administration as well as determining the appropriate moment to start and stop the prophylactic treatment so that the risk of thrombosis becomes minimal.

The secondary objectives set included:

- identification of the prevalence of thromboembolic diseases in the pediatric population studied
- identifying a correlation between high homocysteine levels and an increased risk of thrombosis in children with thromboembolism and hyperhomocysteinemia.
- identification of a correlation between deep vein thrombosis related to the presence of the central venous catheter and the presence of genetic factors
- adapting the treatment to individual needs – identifying the treatment used in cases of thromboembolism, the advantages and disadvantages of each preparation as long-term prophylaxis.
- identifying a correlation between contraceptive administration and thrombotic events in adolescents
- identification of a correlation between the presence of migraine and the polymorphism of the MTHFR gene.

The working hypotheses were as follows:

1. Having a protocol for the diagnosis, treatment and monitoring of pediatric patients with thrombophilia improves the long-term prognosis of patients with thrombophilia

2. MTHFR polymorphisms do not correlate with hyperhomocysteinemia

3. MTHFR polymorphisms do not pose a risk factor for deep vein thrombosis

We conducted an analytical, prospective, observational study, the enrollment period being between May 2020 and May 2023. The follow-up period was from one year to 4 years from the date of diagnosis, until the end of May 2024. Over the course of 36 months, 70 pediatric patients with thrombophilia and thrombotic events or with the suspicion of a thrombotic event were monitored in the Fundeni Cynical Institute.

The patients in the study were evaluated, as appropriate, by the pediatric neurologist, cardiologist or cardiovascular surgeon, received emergency treatment according to specific protocols, were evaluated by imaging (Doppler or MRI, as appropriate), and were subsequently redirected to the pediatric hematologist to identify if there was a pre-existing cause that favored the occurrence of the thrombotic event.

These patients had blood samples taken approximately 3 months after the thrombotic event, in order to dose the biomarkers considered, and were reevaluated at 3, 6 months or annually, depending on the severity of the thrombotic event and the treatment performed.

Target group and inclusion criteria - children who presented the following:

- Purpura fulminans
- infants with spontaneous venous thromboembolism
- Children or adolescents with spontaneous/relapsed VTE or associated with another transient risk factor (not related to the presence of a central venous catheter)
- children or adolescents with arterial thrombosis
- Children undergoing chemotherapy treatment (L-asparaginase)
- asymptomatic children with significant hereditary-collateral history (parents with thrombophilia)

Baseline assessment of all patients who entered the study

Patients have not been examined paraclinically:

- during an acute episode (apart from purpura fulminans)
- not during periods when they are receiving anticoagulant treatment with heparin or dicoumarin (warfarin, acenocoumarol)
- less than 2 days after stopping heparin or 15 days after stopping dicoumarinics
- less than 30 days after stopping hormonal treatment
- no earlier than 90 days postpartum (in the case of pregnant adolescents)

- Tests were performed:
- at least 3 months after a thrombotic event
- in case of interpretation of the results in infants up to 6 months, normal values of haemostasis factors for each age stage were taken into account
- in case of confirmation of a positive result for hereditary thrombophilia, parental testing was also considered (Marcu AM, 2023)

The database obtained by entering this information was processed with the help of a statistical analysis program.

Summary of chapters

The present paper is structured in two parts, the current scientific context and the personal study, and is based on bibliographic references from international specialized publications.

In the first chapter of the doctoral thesis, theoretical data on thrombophilia in children are presented, including epidemiology, etiology and risk factors, elements for establishing a prompt, correct and complete diagnosis, information related to the possibilities of paraclinical testing through biochemical, immunological, coagulological and genetic tests. Also, the methods of diagnostic and imaging monitoring of thrombotic events are exemplified, both those used in daily practice and those less accessible to the pediatric hematologist.

The second chapter of the theoretical part details the problem of treatment in this pathology, from the intricate mechanisms of action of each pharmacological preparation to the indications for use, doses, side effects, advantages and disadvantages of their administration. At the end of this chapter, some lifestyle recommendations are listed that should be addressed by the pediatric patient with thrombophilia who has gone through a thrombotic event.

In Chapters 3-5 of the special part, in addition to the motivation, purpose and objectives of choosing the studied topic, the results of the personal study are presented, exemplified with tables and graphs. We followed the demographic parameters (age, sex), risk factors, hematological and biochemical parameters (coagulogram represented by antithrombin III, protein C, protein S, FVIIIa, APTT, INR, lupus anticoagulant, determination of antiphospholipid, anticardiolipin, anti-beta 2 glycoprotein 1 antibodies, homocysteine, folate and vitamin B12 values), clinical manifestations for which the patient was referred to pediatric

hematology for evaluation and monitoring, treatment followed and its duration, as well as correlations between these criteria.

Subsequently, based on the results obtained, we discussed the objectives of the thesis in turn, compared to current works in the literature.

In Chapter 6 – Discussions – we described the sample analyzed in the study in terms of demographic data 70 patients aged between 2 months and 17 years, with a mean age of 8.47 years, with a median of 9 years, with two peaks of age ranges, most of them being in the age groups 1-6 years (36.2%) and 11-18 years (40.6%).

From the point of view of the distribution of patients in relation to sex, the data from the present study identify a ratio between the sexes is approximately 1:1.5, with 38.60% female patients compared to 61.40% male patients, the age of the patients not being significantly different in relation to sex (an average of 9.21 years for girls and 8.03 years for boys).

Following the study, we identified the prevalence of thromboembolic disorders in the pediatric population studied, with the mention that these findings are obtained from a small, unicenter study and may not be widely applicable.

In terms of the incidence of thrombotic events analyzed, most of the patients included in the study had a previous stroke episode of 35.7%, followed by DVT – 17.1%, TIA – 5.7%, PET 1.4%.

o Correlated the distribution of patients in relation to age category and the existence of DVT, the differences observed between groups were not significantly different according to the Fisher test ($p=0.057$), but there was a tendency towards statistical significance in the direction of associating patients aged 11-18 years more frequently with deep vein thrombosis (75% vs. 33.3%).

o Also, regarding the comparison of patients' age in relation to the existence of stroke, the age distribution in the case of patients without stroke was non-parametric according to the Shapiro-Wilk test ($p<0.001$). The differences observed between groups were statistically significant according to the Mann-Whitney U test ($p=0.015$), patients who had stroke had a significantly younger age (median = 6 years, IQR = 3-9 years) compared to patients without stroke (median = 11.5 years, IQR = 3.25-15 years).

o The differences observed between the groups were significantly different according to the Fisher test ($p<0.001$), and the Z tests with Bonferroni correction showed that patients

aged 7-10 years were significantly more frequently associated with stroke (36% vs. 6.8%) while patients aged 11-18 years were significantly less associated with stroke at presentation (56.8% vs. 12%).

Regarding the distribution of patients related to the existence of central venous catheter (CVC) use of the type of thrombosis and associated mutations, correlations were identified between deep vein thrombosis related to the presence of central venous catheter and the presence of genetic factors. The results showed the following:

o The frequency of central venous catheter use was not significantly different in relation to the existence of DVT (p=1,000) and PET (p=1,000), but in the case of stroke patients, patients who had CVC had significantly more frequent stroke at presentation (12% vs. 0%) (p=0.042);

o The frequency of use of the central venous catheter was not significantly different in relation to most of the associated mutations (p>0.05), except for the prothrombin mutation where a trend towards statistical significance was observed (p=0.056), in the direction of the more frequent presence of the mutation in patients who used CVC (22.2% vs. 2%).

We could not identify a statistically significant correlation between contraceptive administration and thrombotic events in adolescents, but from the study of the distribution of patients in relation to the existence of migraine and risk factors, we concluded that the differences observed between groups were not significantly different according to the Fisher tests (p>0.05), with the exception of testing the association between the existence of migraine and contraceptive use (p=0.049), where patients who used contraceptives were significantly more frequently associated with migraines (12.5% vs. 0%).

Regarding the identification of a correlation between the presence of migraine and the polymorphism of the MTHFR gene, we obtained the following results:

o 16 patients (22.9%) presented with migraines and the differences observed between groups were statistically significant according to the Mann-Whitney U test (p<0.001), patients who had migraines had a significantly higher age (median = 15 years, IQR = 9-16 years) compared to patients without migraines (median = 6 years, IQR = 2-12.25 years).

o The differences observed between groups were significantly different according to the Fisher test (p=0.009), and the Z tests with Bonferroni correction showed that patients aged 1-6 years were significantly less associated with migraines (44.4% vs. 6.7%) while patients aged

11-18 years were significantly more frequently associated with migraines at presentation (73.3% vs. 31.5%).

MTHFR polymorphisms do not present a risk factor for deep vein thrombosis and do not statistically correlate with hyperhomocysteinemia.

Methylenetetrahydrofolate reductase (MTHFR) polymorphisms, specifically C677T and A1298C, were once considered potential risk factors for thrombosis due to their association with high levels of homocysteine, a condition known as hyperhomocysteinemia. However, recent studies have changed this perspective, and MTHFR polymorphisms are no longer considered significant risk factors for thrombosis for the following reasons:

- weak association with thrombosis: large-scale studies and meta-analyses have shown that the association between MTHFR polymorphisms and venous thrombosis is either very weak or non-existent. For example, the MEGA study, which involved more than 4,000 patients, found no significant association between the MTHFR 677C>T polymorphism and the risk of venous thrombosis (Bezemer et al., 2007).

- no causal link to increased homocysteine – while MTHFR polymorphisms may result in slightly elevated homocysteine levels, studies have not shown a direct causal link between these polymorphisms, increased homocysteine, and an increased risk of thrombotic events. The conclusion is that mild hyperhomocysteinemia, caused by MTHFR polymorphisms, does not independently lead to thrombosis (Gouveia & Canhão, 2010).

In the study, out of a total of 70 patients, the MTHFR C677T polymorphism was identified in 33 patients (50%), of which 26 had heterozygous status (78.78%) and 7 homozygous status (21.22%). MTHFR A1298C polymorphism was identified in 36 patients (54.5%), of whom 30 (83.33%) were heterozygous and 6 (16.67%) were homozygous.

It was identified that, in patients with the presence of the MTHFR C667T polymorphism, the mean homocysteine values were similar to those where the presence of this polymorphism was not identified (10.88 umol/L versus 9.07 umol/L), most homocysteine values being normal.

Only in the case of 10 patients were homocysteine values above 13 umol/L, but in almost equal proportions for the two categories of patients studied (4 children with thrombosis and the presence of MTHFR polymorphism – 6 children with thrombosis without the presence

of MTHFR polymorphism). We can conclude that the frequency of increased homocysteine was not significantly different in relation to the presence of polymorphism.

Regarding the identification of a correlation between high homocysteine levels and an increased risk of thrombosis in children with thromboembolism and hyperhomocysteinemia, the measurements were not statistically significant but, analyzing the comparison of the evolution of homocysteine at diagnosis at 3 months in patients with a homocysteine value at diagnosis greater than or equal to 13 μmol/L, After administration of oral folic acid treatment, we highlighted that the distribution of the differences observed between measurements were statistically significant according to the Paired-Samples T-Test ($p<0.001$), observing a significant decrease in homocysteine from diagnosis (17.64 ± 3.4 μmol/L) to the value at 3 months (9.26 ± 1.95 μmol/L), the difference recorded being significant (8.375 μmol/L, 95% C.I.: 5,642-11,108).

Based on the data obtained from the study, I recommend the following protocol for the diagnosis, treatment and monitoring of a pediatric patient with thrombophilia after an acute thrombotic event, with the mention that genetic testing does not influence the management of the acute thrombotic episode itself, but may be important for determining the duration of thromboprophylaxis in the long term.

1. Identification of the type of thrombosis and its location (venous/arterial, peripheral, cerebral or with unconventional location)

2. Identification of the time of the thrombotic event in order to choose the optimal time for the dosage of specific biological markers. It is recommended to limit their dosage during the acute episode, as it is known that some of them can be falsely modified (they act similarly to acute phase reactants, such as increased coagulation factor VIII) and do not represent the patient's baseline. Patients will not be examined paraclinically:

- During an acute episode (apart from purpura fulminans);
- during periods when they receive anticoagulant treatment with heparin or dicoumarin (warfarin, acenocoumarol);
- Less than 2 days after stopping heparin or 15 days after stopping dicoumarins;
- Less than 30 days after stopping hormone treatment;
- No earlier than 90 days postpartum (in the case of pregnant adolescents);

1. Identification of the thrombosis character:

- idiopathic thrombotic event (no obvious clinical risk factor can be identified)
- nonidiopathic
- provoked thrombotic event (trauma, surgery, dehydration, pregnancy)
- unprovoked thrombotic event;

In case of identifying a triggering factor, recording it in order to try to avoid it in the future or, if it cannot be avoided, taking preventive measures to avoid the recurrence of the thrombotic event.

2. Identification of associated risk factors (persistent, permanent or quasi-permanent factors): obesity, hypertension, diabetes, kidney disease, malignancies, immobilization, administration of contraceptives or hormone replacement therapy, anatomical vascular peculiarities, smoking.

3. Identification of hereditary-collateral antecedents. A positive family history of venous thrombosis may reflect the presence of genetic risk factors in a family. Carriers of a genetic risk factor are at increased risk of first venous thrombosis, especially when exposed to environmental triggers and an increased risk of recurrence. Although family history cannot be used to identify genetic risk factors because the positive predictive value and sensitivity are low, it can guide the clinician in making the genetic testing decision. In the case of genetic testing, knowledge of pre-existing mutations can help to choose the test panel (a standard panel or an extended panel).

4. Carrying out basic laboratory investigations:

- Blood count
- Biochemistry for evaluation of liver function, renal function, infectious parameters, LDH, folate, vitamin B12, homocysteine
- Coagulation: aPTT, PT, INR, Ddimers, fibrinogen, level FVIII, to which can be added: level AT III, PC, PS, APCR, lupus anticoagulant
- Anticardiolipin antibodies, anti-beta 2 glycoprotein I antibodies

5. Genetic testing – in selected cases:

- Factor V Leiden – if APCR values are changed
- Prothrombin mutation G20210A

- Congenital deficiency of protein C, protein S or antithrombin – in the case of newborns with purpura fulminans or family cases of deficiency of these proteins with a history of venous thromboembolism.
- PAI -I, EPCR
- WES (low specificity) or extended panels for specific point mutations

6. Choice of treatment based on:
    - Age (some oral anticoagulants cannot be administered to children under 8 or 12 years of age, in some adolescents compliance with treatment may decrease if they require repeated monitoring or chronic administration over a very long period);
    - Need and possibilities for monitoring (aPTT, INR, factor Xa level);
    - Route of administration (continuous, subcutaneous, oral venous infusion);
    - Possible side effects (hypersensitization reactions, heparin resistance, skin necrosis after administration of acenocoumarol or warfarin);
    - Complications that may occur (major or minor hemorrhages, heparin-induced thrombocytopenia, liver cytolysis syndrome that can go up to liver failure, kidney failure);
    - Availability of treatment
    - Drug interactions (in the case of patients with chronic diseases or malignancies, it is recommended to choose the preparation with the fewest pharmacological interactions) or food interactions (in the case of administration of vitamin K antagonists that may require increasing doses if the food received also contains vitamin K, for example some powdered milk formulas or some vegetable formulas);
    - Patient status - inpatient or outpatient
7. Expected duration of thromboprophylaxis:
    - short period, limited by the duration of action of a possible trigger, e.g. treatment with L-asparaginase, surgery, immobilization (orthopedic interventions with plaster cast) or limited possibilities of movement (long flights);
    - average periods – 3-6 months, for provoked thrombotic events, with or without associated risk factors
    - long or indefinite periods, when there are genetic risk factors associated with associated risk factors, of a permanent nature and minimal a thrombotic event in the past, and especially after a recurrent thrombotic event.

8. Available drugs and their indications:
    - Unfractionated heparin – intravenous administration in the acute episode, after excluding an associated hemorrhage, for a duration of approximately 5 days (and to limit the occurrence of heparin-induced thrombocytopenia that is triggered 5-10 days after the initiation of therapy); requires frequent monitoring via aPTT, with dose adjustment as needed. It can be administered from the first days of life, without known important drug interactions, has a safe, predictable pharmacokinetic profile, short half-life of approximately 1.5h. In case of overdose, he has an antidote – protamine sulfate;
    - Low molecular weight heparin - subcutaneous administration as a curative treatment twice a day or prophylactically, once a day, for a short duration (in case of surgery, immobilization) or medium duration (3-6 months in the case of cerebral or deep peripheral venous thrombosis). Lower risk of heparin-induced thrombocytopenia due to the structure of the molecule. It requires monitoring in the first days after initiation of treatment by dosing the anti-factor Xa activity. It can be administered from the first days of life, with the mention that higher doses are needed for infants under 2 months. It has no known important drug interactions, has a safe pharmacokinetic profile, with a half-life of 3-7 h. In case of overdose, protamine sulphate at a dose of 1:1 (1 mg protamine to 1 mg enoxaparin) may be used.
    - Vitamin K antagonists – in our country, the most commonly used is acenocoumarol, with oral administration once a day. It can be used as a medium-long term (indefinite) prophylaxis. It requires frequent monitoring to adjust doses to maintain an INR in the therapeutic range 2-3. It has numerous drug (antibiotics, anticonvulsant medication) and food interactions, a half-life of 8-11 hours, up to 40 hours. Increased risk of bleeding, skin necrosis or osteoporosis in the long term. In case of overdose, vitamin K can be administered intravenously, associated as the case may be with CPP or prothrombin complex concentrate;
    - Direct thrombin inhibitors – dabigatran etexilate is a molecule recently approved for pediatric thromboprophylaxis, in children older than 3 months; oral administration, half-life of approximately 13h, plasma peak at 2h after administration; dosage according to age and weight; has no known antidote in case of hemorrhage, some studies suggest the use of F VIIa.

- Factor Xa inhibitors – rivaroxaban – approved for the treatment of patients with heparin resistance and for thromboprophylaxis after a thrombotic event; hepatic metabolism, numerous drug interactions; half-life of 5-9h; caution is recommended in patients with hepatic cytolysis or hepatic impairment (may increase the cumulative risk of bleeding); dose dependent on age and weight, ranges from 2.5 mg twice a day to 20 mg per day; does not require monitoring, but in special cases an anti-factor Xa level can be dosed with the calibrated device for rivaroxaban; does not have a specific antidote, in case of bleeding it is recommended to administer CPAP.
- Antithrombin III concentrate – only in selected cases, under medical observation, intravenous administration
- Alteplase – intravenous administration, only in selected cases, for thrombolysis in acute episodes and for occluded catheters, under medical observation; half-life between 5 and 72 minutes, low risk of bleeding due to rapid hepatic metabolism.

9. Imaging evaluation of the patient in order to monitor the degree of resolution of the thrombosis and to identify a possible recurrent event, by:
    - Doppler echo if he has been diagnosed with a peripheral deep vein thrombosis
    - imaging methods with higher sensitivity, such as MRI or CT, CT angiography or MRI angiography
    - Pulmonary infusion scintigraphy

From the point of view of the limitations of the study, it is important to mention that the study is single-center, with a small number of patients included in the evaluated group, which determines the decrease in the value of statistical significance. The findings of a small, single-center study may not be widely applicable. The specific population studied may have unique characteristics that are not representative of the general pediatric population with thrombophilia, limiting the ability to generalize the results to other settings or populations (Ruud et al., 2002).

- A small sample size reduces the power of the study to detect significant associations or differences between groups. This can lead to inconclusive or statistically insignificant results, making it difficult to draw solid conclusions. That is why extensive studies are needed, in larger batches, representative of the population studied.

- In small populations, rare results may not be seen at all, making it difficult to understand the full spectrum of complications associated with thrombophilia in children (Kenet et al., 2011).

Also, for financial reasons and the absence of laboratory methodology, some biomarkers were excluded (lipoprotein a assay, testing for other genetic mutations such as factor XIII mutation, PAI-I);

Limited access to Doppler ultrasound for vascular evaluation (few pediatric specialists, long waiting time, need to perform the evaluation by the same person each time).

It is important to emphasize the need to carry out batch studies with a larger number of children with this pathology, in order to determine the most precise duration of the anticoagulation period, the choice of the most appropriate drug for the type of thrombosis, taking into account its severity, the risk of recurrence, the patient's age and the route of administration.

It may be useful to determine several biomarkers related to the occurrence of thrombosis in the pediatric population, in order to make correlations as precise as possible related to the early identification of thrombotic risk.

It is necessary to develop the possibility of genetic testing, both for known mutations and polymorphisms, as well as for the rarest ones, in order to be able to advise future parents about the risk of a thrombotic episode, both intrauterine and during the life of the future child.

In Chapter 7 - Conclusions and personal contributions I underlined the fact that my doctoral thesis is the first and only study in Romania conducted so far on a batch of pediatric patients with thrombophilia, the field of pediatric thrombosis being one with many unknown variables to explore.

From the data obtained through this unicentric study, I managed to outline a first protocol for the diagnosis, monitoring and treatment of pediatric patients with thrombosis in the country, which, I hope, will become a basis on which more and more data from future studies and clinical research can be added, so that the treatment of thrombosis occurring at any age can be managed by both pediatric hematologists, and those in related specialties.

Since in our country there are currently no protocols for anticoagulation and follow-up of pediatric patients diagnosed with thrombophilia as a result of a thrombotic event, I recommend that the evaluation of the pediatric patient with a provoked or unprovoked thrombotic episode contain a complete and correct anamnesis of both the patient and his relatives in order to identify hereditary-collateral risk factors, a minimum dosage of the available biomarkers, with the understanding of the importance of the moment of determining

each biomarker in part, the changes that occur can be determined by the presence of acute thrombosis and not actually representing the normal baseline of the patient.

Pediatric hematology is a challenging specialization. New emerging pathologies in the pediatric population require innovation both from the point of view of the medical approach and from the point of view of the pharmacological approach. The concept of "personalized therapy" is gaining momentum in terms of approaching children. Personalized therapy for pediatric patients involves tailoring medical treatments to the individual characteristics of each child, which may include genetic makeup, developmental stage, and environmental factors. This approach aims to improve the effectiveness of treatment and minimize side effects by taking into account factors unique to each patient.

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