SEXUAL MEDICINE REVIEWS REVIEW

Testosterone Therapy: An Assessment of the Clinical Consequences of Changes in Hematocrit and Blood Flow Characteristics

Carola S. König, PhD,[1] Stavroula Balabani, PhD,[2] Geoffrey I. Hackett, MD,[3] Richard C. Strange, PhD,[4] and Sudarshan Ramachandran, PhD, FRC Path[1,5,6]

ABSTRACT

Introduction: Clinical guidelines indicate that hematocrit should be monitored during testosterone replacement therapy (TTh), with action taken if a level of 0.54 is exceeded.

Aim: To consider the extent of changes in hematocrit and putative effects on viscosity, blood flow, and mortality rates after TTh.

Methods: We focused on literature describing benefits and possible pitfalls of TTh, including increased hematocrit. We used data from the BLAST RCT to determine change in hematocrit after 30 weeks of TTh and describe a clinical case showing the need for monitoring. We consider the validity of the current hematocrit cutoff value at which TTh may be modified. Ways in which hematocrit alters blood flow in the micro- and macro-vasculature are also considered.

Main Outcome Measures: The following measures were assessed: (i) change in hematocrit, (ii) corresponding actions taken in clinical practice, and (iii) possible blood flow changes following change in hematocrit.

Results: Analysis of data from the BLAST RCT showed a significant increase in mean hematocrit of 0.01, the increase greater in men with lower baseline values. Although 0 of 61 men given TTh breached the suggested cutoff of 0.54 after 30 weeks, a clinical case demonstrates the need to monitor hematocrit. An association between hematocrit and morbidity and mortality appears likely but not proven and may be evident only in patient subgroups. The consequences of an increased hematocrit may be mediated by alterations in blood viscosity, oxygen delivery, and flow. Their relative impact may vary in different vascular beds.

Conclusions: TTh can effect an increased hematocrit via poorly understood mechanisms and may have harmful effects on blood flow that differ in patient subgroups. At present, there appears no scientific basis for using a hematocrit of 0.54 to modify TTh; other values may be more appropriate in particular patient groups. **König CS, Balabani S, Hackett GI, et al. Testosterone Therapy: An Assessment of the Clinical Consequences of Changes in Hematocrit and Blood Flow Characteristics. Sex Med Rev 2019;XX:XXX–XXX.**

Key Words: Adult-Onset Testosterone Deficiency; Testosterone Therapy; Hematocrit; Type 2 Diabetes; Blood Viscosity; Microvascular Flow

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[1]College of Engineering, Design & Physical Sciences, Brunel University, London, England, United Kingdom;

[2]Faculty of Engineering Sciences, University College London, London, United Kingdom;

[3]Department of Urology, University Hospitals Birmingham NHS Foundation Trust, West Midlands, England, United Kingdom;

[4]Institute for Science and Technology in Medicine, Keele University, Staffordshire, England, United Kingdom;

[5]Department of Clinical Biochemistry, University Hospitals Birmingham NHS Foundation Trust, West Midlands, England, United Kingdom;

[6]Department of Clinical Biochemistry, University Hospitals of North Midlands / Faculty of Health Sciences, Staffordshire University, Staffordshire, England, United Kingdom

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INTRODUCTION

Use of testosterone therapy (TTh) is increasing rapidly worldwide. For example, in the United States the cost of TTh in 2013 was $2.4 billion with an expected increase to $3.8 billion in 2018 (https://www.statista.com/statistics/320301/predicted-annual-testosterone-drug-revenues-in-the-us/ - accessed on 01/11/2018). Although the benefits of TTh in terms of reduced risk of cardiovascular disease (CVD) and mortality are reported in an increasing number of studies, some reports present a contrary view. This review is in 2 sections; the first considers the merits of TTh in men with low serum testosterone and associations between increased hematocrit, a well-recognized adverse effect of the therapy, and morbidity/mortality. The second part considers the association between hematocrit and blood flow and discusses

putative mechanisms whereby TTh may lead to development of pathology. Our approach has been to review the literature and present unpublished data from our BLAST randomized controlled trial that describes the relationship between TTh, increased hematocrit, morbidity and mortality. The importance of monitoring hematocrit is emphasized in a case report showing TTh leading to a markedly increased hematocrit requiring phlebotomy and replacement of injectable TTh with a gel formulation. We also speculate on the effect that increased numbers of red blood cells could have on blood flow characteristics in different vascular beds. The review is based on publications from basic science, longitudinal and randomized controlled trials, and reviews known to us or selected from PubMed (US National Library of Medicine).

TESTOSTERONE DEFICIENCY

Testosterone deficiency (TD), characterized by low testosterone levels and related symptoms, occurs in 6-12% of men.[1,2] TD is associated with decreased bone mineral density, lean mass, muscle strength, cognitive function, sexual function, and increased fat mass.[1–3] The phenotype is categorized as primary TD, secondary TD (pituitary/hypothalamic disease), or adult-onset TD. Adult-onset TD describes, in men older than 50 years (following exclusion of hypothalamic-pituitary-testicular axis pathology), a combination of low serum testosterone levels and accompanying symptoms. The condition is associated with obesity, type 2 diabetes mellitus (T2DM), and the metabolic syndrome (MetS).[3,4] Prevalence of adult-onset TD in men with T2DM is about 40%.[5,6] Indeed, low testosterone levels are associated with the number and severity of components classifying the MetS (increased waist circumference/body mass index, glycemia, triglycerides, blood pressure, and decreased high-density lipoprotein cholesterol) and may also predict the onset of diabetes in younger men.[7]

Importantly, adult-onset TD is associated with increased morbidity and mortality rates.[4] For example, the European Male Ageing study, (2,599 men, 7% with T2DM, aged 40−79 years, about 4 years' follow-up) showed that the combination of TD symptoms and total testosterone <8 nmol/L (230.5 ng/dL) was significantly associated with increased total and CVD-related mortality.[8] Importantly, there is also accumulating evidence from longitudinal observational studies that TTh can lead to improved sexual health and reduced all-cause mortality rates.[4] Shores et al[9] studied the impact of TTh on mortality rates in 1,031 men (aged >40 years, total testosterone ≤ 8.7 nmol/L [250.7 ng/dL], mean follow-up about 4 years); the mortality rate in 398 men on TTh was 10.3%, compared with in untreated control subjects (20.7%). Survival analysis showed significantly reduced mortality rates in men with T2DM but not in their non-diabetic counterparts. This finding was confirmed by 2 longitudinal studies of men with T2DM. Muraleedaran et al[10] studied, over 6 years, the effects of low testosterone (the cohort stratified by total testosterone of 10.4 nmol/L [299.7 ng/dL]) and TTh on mortality in 581 men with T2DM. The mortality rate was higher in 238 men with low testosterone (hazard ratio [HR] = 2.02, 95% CI = 1.2−3.4), compared with those with values >10.4 nmol/L (299.7 ng/dL) after adjustment for confounders. In the low-testosterone group, the 174 men not on TTh were at significantly higher risk of death (HR = 2.3, 95% CI = 1.3−3.9) than the 64 men receiving TTh. A longitudinal study in 857 men with T2DM by our group showed similar results.[11] We stratified the cohort using a total testosterone cutoff of 12.0 nmol/L (345.8 ng/dL) and free testosterone of 0.25 nmol/L (7.2 ng/dL); over a mean 3.8 years, the mortality rate was reduced in men on TTh, with greatest benefit in older men.[11,12] Survival analysis (adjusted for age, phosphodiesterase 5-inhibitor, and statin treatment) showed that, compared with men with low testosterone (either low total or calculated free testosterone) not on TTh, the mortality rate was lower in men with normal testosterone (HR = 0.62, CI = 0.41−0.94) and men with low testosterone on TTh (HR = 0.38, CI = 0.16−0.90). This benefit was independent of changes in conventional cardiovascular/metabolic risk factors (weight, body mass index [BMI], dyslipidemia, glycemic control, blood pressure).[13] Furthermore, Snyder et al[14] in the Testosterone trial, showed significant benefits in sexual function, mood, depression, quality of life, physical performance, vitality, anemia, and bone mineral density in the overall group, although this benefit was not evident in the individual studies. The BLAST randomized controlled study suggested improvements in erectile dysfunction after TTh, especially in men with total testosterone levels <8 nmol/L (230.5 ng/dl); the change reached statistical significance only after 6 months of therapy,[15,16] with improvement continuing even after 4 years.[17]

The above data provide the basis for the British Society for Sexual Medicine[4] and International Society for Sexual Medicine guidelines (https://professionals.issm.info/wp-content/uploads/sites/2/2018/05/ISSM-Quick-Reference-Guide-on-TD.pdf - accessed on 02/01/2019) that include the following management recommendations:

- Total testosterone <8 nmol/L (230.5 ng/dL) or free testosterone <0.180 nmol/L (5.2 ng/dL); usually requires TTh.
- Total testosterone >12 nmol/L (345.8 ng/dL) or free testosterone >0.225 nmol/L (6.5 ng/dL); does not require TTh.
- Total testosterone 8−12 nmol/l (230.5−345.8 ng/dL) may require a trial of TTh for a minimum of 6 months depending on symptoms.

POSSIBLE CARDIOVASCULAR ADVERSE EFFECTS ASSOCIATED WITH TTH

Concern continues to exist (https://www.fda.gov/Drugs/DrugSafety/ucm436259.htm - accessed on 02/01/2019) (http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Testosterone_31/Recommendation_provided_by_Pharmacovigilance_Risk_Assessment_Committee/WC500175213.pdf - accessed on 02/01/2019) regarding the cardiovascular safety of TTh in the treatment of adult-onset TD. Thus, whereas most studies demonstrate either benefit or no increase in

cardiovascular events, a few widely cited studies have reported increased CVD in patients on TTh.[18,19] Vigen et al[20] used a composite of all-cause mortality, myocardial infarction, and stroke rates as outcome in patients with low testosterone levels who had undergone coronary angiography and subsequently received TTh. Although the event rate was 10.1% in testosterone-treated and 21.2% in untreated patients, after adjustment for >50 variables (baseline testosterone and erectile dysfunction, both associated with all-cause mortality, were not included), TTh appeared to be associated with increased events (25.7% in treated, 19.9% in untreated groups) over 3 years' follow-up. Finkle et al[21] examined 55,593 insurance claims and compared the incidence rate of myocardial infarction in the 12 months before and 3 months after the initial prescription of TTh and reported an increased rate of non-fatal myocardial infarctions, especially in men aged ≥65 years. In younger men, the risk was confined to those with pre-existing heart disease. Importantly the control group was comprised of men commenced on phosphodiesterase 5-inhibitors, which have been demonstrated to lower cardiovascular and all-cause mortality rates[11,12,22,23]. Furthermore, there were design flaws, including lack of data on testosterone levels, the reporting of only non-fatal events, and a retrospective review of the previous 12 months only after the decision on TTh was made. 3 months' follow-up may be insufficient to detect benefit from TTh, and the authors conceded that increased events could be related to TD rather than TTh. Basaria et al[24] published the Testosterone in Older Men with Mobility Limitations Trial of 209 older men (mean age 74 years), with limited mobility randomized to either testosterone or placebo gel. Although the primary outcome (change in maximal voluntary muscle strength during leg press exercise) was met, the trial was discontinued because 23 men given TTh and 5 men given placebo seemed to develop cardiovascular-related adverse events. However, in addition to the limitations imposed by a relatively small cohort, there was no cardiovascular assessment at baseline, and events were based on self-reporting and included a wide range of symptoms, including peripheral edema and syncope. Although these studies have flaws, and the mechanism for the putative adverse effect of TTh is unidentified, they do indicate a need to better assess potential problems in using TTh, especially in patient subgroups.

We recently speculated on the effects that patient heterogeneity could have on treatment benefits.[25] For example, a small patient subgroup may be at an increased risk that cannot be detected in large trials with wide inclusion criteria. Accordingly, clinical outcomes associated with TTh need to be identified using randomized controlled trials and observational longitudinal studies that evaluate benefits and adverse effects in the total cohort and subgroups.

INCREASED HEMATOCRIT AFTER TTH

In this context, the effects of TTh on hematocrit and potentially clinical outcomes are important. Thus, an increase in hematocrit is the commonest adverse effect linked with TTh,[26–28] with values >0.54 used to alter dose or discontinue treatment.[4]

The need for regular monitoring of hematocrit is exemplified in a case report. A 53-year-old man was referred to the metabolic clinic at University Hospitals Birmingham NHS Foundation Trust in 2007 with severe fatigue, erectile dysfunction, and the MetS. He was diagnosed with T2DM in 2012, with a total testosterone of 3.6 nmol/L (103.7 ng/dL), luteinizing hormone = 0.6 IU/L, follicle stimulating hormone = 3.6 IU/L, and similar values on repeat testing (fasting sample at 9 AM). Other biochemical investigations, including a GnRH dynamic function test, were unremarkable. A diagnosis of adult-onset TD was made, and the patient was commenced on testosterone gel (2%) with 4 applications/day (each application 10 mg testosterone), with hematocrit levels <0.54 (0.499–0.536) until November 2016 (total testosterone: 6.9 nmol/L [198.8 ng/dL], calculated free testosterone: 0.14 nmol/L [4.0 ng/dL]). When the testosterone gel was increased to 5 daily applications in view of persisting symptoms, the hematocrit increased to 0.565 (total testosterone: 12.0 nmol/L [345.8 ng/dL]), calculated free testosterone: 0.24 nmol/L [6.9 ng/dL]). TTh was reduced to 4 applications daily, and, in view of increasing fatigue levels, the patient was referred to the Urology Clinic in January 2017, and testosterone undecanoate injections were commenced in March 2017. Energy levels improved with this therapy, and in June 2017 total testosterone was 13.3 nmol/L (383.3 ng/dL), calculated free testosterone was 0.25 nmol/L (7.2 ng/dL), and hematocrit was 0.536. The hematocrit had significantly increased at review in December 2017; total testosterone = 28.8 nmol/L (830.0 ng/dL), calculated free testosterone = 0.70 nmol/L (20.2 ng/dL), and hematocrit = 0.638. The testosterone undecanoate was immediately discontinued, and the patient commenced on aspirin. A check after 3 weeks showed the hematocrit was 0.648, serum total testosterone was 26.8 nmol/L (772.3 ng/dL), and calculated free testosterone was 0.65 nmol/L (18.7 ng/dL). The patient underwent immediate venesection, and the hematocrit gradually reduced: January 2018 = 0.557; February 2018 = 0.544; April 2018 = 0.537; July 2018 = 0.530. In August 2018, the hematocrit was 0.499, with total testosterone of 3.7 nmol/L (106.6 ng/dL) and calculated free testosterone of 0.08 nmol/L (2.3 ng/dL). The patient was suffering severe fatigue and erectile dysfunction and wished to restart testosterone gel. At the last follow-up in August 2018 (on 4 gel applications) the hematocrit was 0.507, total testosterone 15.4 nmol/L (443.8 ng/dL), and calculated free testosterone was 0.41 nmol/L (11.8 ng/dL) with some clinical improvement.

The patient's written consent allowing us to describe this case was obtained and filed in his hospital notes. It emphasizes the need for monitoring and appropriate action where needed. As recommended by guidelines, we used a hematocrit of 0.54 as threshold for reducing or stopping TTh.[4] This level appears

based on the hematocrit reference range and not on evidence. Clearly, given data linking elevated hematocrit levels with increased morbidity/mortality rates, an evidence-based threshold value that can be used in clinical practice is needed. A further issue in using TTh may be the testosterone preparation and its mode of delivery.[28] A recent comprehensive review suggested short-acting, injectable testosterone is associated with greater risk of elevated hematocrit compared with other preparations.[28] This raises the possibility that the rate of change in serum testosterone concentration may be mechanistically important; short-acting injectable testosterone could lead to steeper rises and falls in hormone levels that in turn has effects on erythrocytosis. However, as demonstrated by the above case, monitoring of hematocrit is needed with all testosterone preparations including long-acting injectable TTh.

CHANGE IN HEMATOCRIT AFTER 30 WEEKS TTH IN THE BLAST STUDY

The BLAST randomized controlled trial (European Union Clinical Trials Register: EudraCT 2008-000931-16) comprised a 30 week, randomized, double-blind, placebo-controlled, multicenter study carried out during September 2008−June 2012 to assess the impact of TTh using testosterone undecanoate, a long-acting injectable preparation in 199 men with T2DM (primary outcome: change in glycemic control).[29] Baseline and final visit hematocrit data were available in 134 (placebo = 73 men, TTh = 61 men) of the 189 men completing the study (placebo = 103 men, TTh = 86 men). No significant change in hematocrit was observed in the placebo group (baseline = 0.432, final visit = 0.435, P [paired t-test] = .22). The hematocrit increased significantly in the TTh group (baseline = 0.444, final visit = 0.454, P [paired t-test] = .01) but did not breach the 0.54 threshold in any patient during 30 weeks of treatment.

Hematocrit at baseline was only associated with diastolic blood pressure (linear regression, coefficient [c]: 0.11, 95% CI = 0.06−0.17, $P < .001$). When adjusted for baseline age, total testosterone and other classifying characteristics of the MetS (body mass index, triglycerides, high-density lipoprotein cholesterol, HBA1c, systolic blood pressure), this association remained significant (multiple regression, c = 0.07, 95% CI = 0.006−0.14, $P = .03$). Only baseline hematocrit (not age, total/free testosterone level or metabolic parameters) was associated with the 0.010 increase in hematocrit in men on TTh (c = −0.35, 95% CI = −0.56 to −0.14, $P = .001$). The finding that the coefficient had a negative value indicates reassuringly, that lower and not higher baseline hematocrit levels were associated with the largest increases. No such association was observed in the placebo group (c = −0.11, 95% CI = −0.29−0.07, $P = .22$). Figure 1 illustrates the change in hematocrit after 30 weeks of treatment with placebo or TTh. The negative coefficient observed between baseline and change in hematocrit in the BLAST patients differs from data reported by Ip et al,[30] who showed higher trough testosterone levels predicted an increased hematocrit >0.50.

The mechanism for increased hematocrit following TTh is unclear. Coviello et al[31] demonstrated a linear dose-dependent increase in hemoglobin and hematocrit levels after TTh; this was observed in both 60 men aged 60−75 years and 61 men aged 19−35 years. However, the increase was more evident in the older men. Interestingly, we observed greater reductions in mortality rates after TTh in older men.[11,12] No increase in erythropoietin or the marker of bone marrow erythropoietic activity, soluble transferrin receptor, was noted. They speculated that androgens may have a direct stimulatory effect on the bone marrow and perhaps promote differentiation of erythroid colony forming units into erythropoietin sensitive cells. In contrast Bachman et al[32] found that an increase in hemoglobin and hematocrit was associated with elevated erythropoietin levels 1−3 months after TTh, but the levels returned to normal after 6 months. Despite increased hemoglobin and hematocrit, erythropoietin levels were not suppressed.

ELEVATED HEMATOCRIT AND INCREASED MORBIDITY/MORTALITY

We now consider longitudinal observational studies that evaluate the association between hematocrit and CVD. Although no consensus has been reached, there are hints of a non-linear relationship. In a meta-analysis of 16 population-based prospective studies comprising 8,020 individuals (mean hematocrit = 0.440), Danesh et al[33] showed that the top tertile of hematocrit (hematocrit > 0.463) was associated with increased coronary heart disease (risk ratio = 1.16, 95% CI = 1.05−1.29), compared with the bottom tertile (hematocrit < 0.417). Addition of another 3 trials comprising individuals with established cardiovascular disease strengthened the above association between the 2 extreme tertiles (risk ratio = 1.81, 95% CI = 1.19−2.76). However, the authors urged caution because adjustment for other coronary heart disease risk factors, which were associated with hematocrit levels, varied between the trials.

These findings were not confirmed by the European Prospective Investigation into Cancer and Nutrition - Netherlands (EPIC-NL) study (derived from the MORGEN-EPIC and Prospect-EPIC studies) comprising 16,187 individuals without CVD at baseline.[34] No association was found between the hematocrit tertiles (cutoff values 0.45 and 0.47) and 10-year risk of CVD, strokes, and coronary heart disease.

The Scottish Heart Health Extended Cohort Study estimated the predictive value of plasma viscosity, hematocrit and whole blood viscosity (dependent on plasma viscosity and hematocrit) for cardiovascular events in 3,386 men and women aged 30−74 years followed up for 10−21 years.[35] High plasma viscosity was independently associated with CVD events and mortality. Although hematocrit (mean ± SD = 0.4381 ± 0.0394) was significantly associated with CVD events (HR = 1.14, 95%

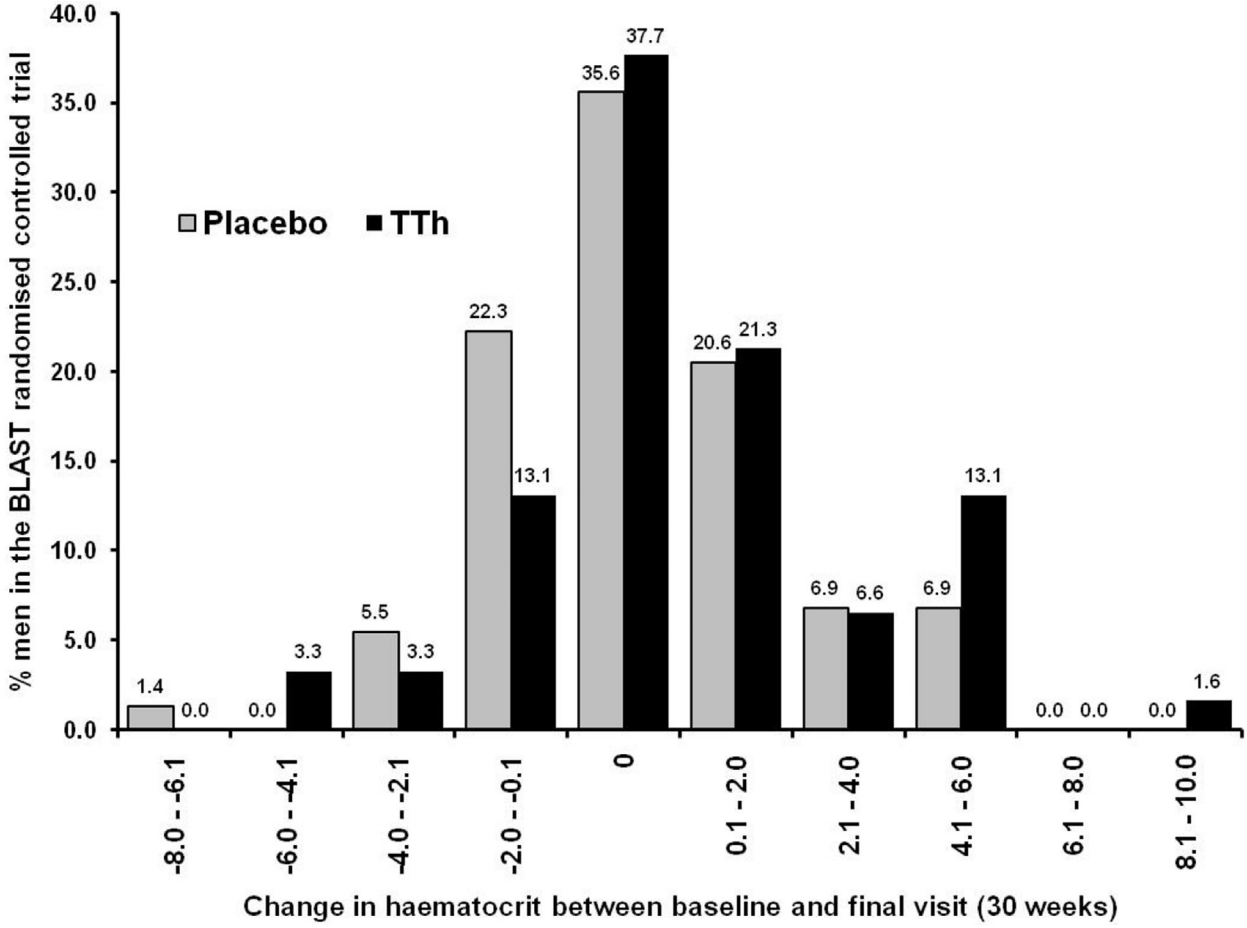

Figure 1. Change in hematocrit after 30 weeks of treatment in the placebo and TTh groups (BLAST study of men with T2DM). TTh = testosterone replacement therapy; T2DM = type 2 diabetes mellitus.

CI = 1.04−1.25, $P = .004$) and mortality (HR = 1.22, 95% CI = 1.11−1.33, $P < .001$) when adjusted only for age and sex, significance was lost when confounders such as lipids, blood pressure, diabetes, smoking status, family history of CVD, and fibrinogen were included.

A 34-year follow-up of 5,209 men and women from the Framingham cohort indicated that the highest hematocrit quintile was associated with increased CVD and all-cause mortality.[36] A dual effect was hinted at with a J or U shaped relationship between hematocrit and cardiovascular events. Further evidence for a non-linear association was added by Boffetta et al[37]; in a study of 49,983 Iranian adults, a U-shaped relationship between categories of hematocrit and mortality was found in both sexes, with both low and high values associated with increased overall mortality. In men, compared with the reference group (hematocrit 0.40−0.44), all-cause mortality and mortality related to CVD were increased when the hematocrit was either <0.39 or >0.45 (adjusted Cox regression), whereas in women, in comparison to the reference group (0.35−0.40), all-cause mortality was greater when hematocrit was <0.35 or >0.40, and mortality related to CVD was greater when hematocrit was <0.30 or >0.40.

Locatelli et al[38] studied the effects of erythropoietin in 5,302 patients with end-stage renal disease (mean baseline hematocrit ± SD = 0.301 ± 0.045) on the Lombardy Registry. It was evident that all-cause mortality risk was inversely proportional to the increase in hematocrit (OR = 0.95, 95% CI = 0.92−0.97) after erythropoietin therapy. It was concluded that a higher hematocrit achieved either spontaneously or after erythropoietin therapy improved outcomes in patients undergoing dialysis. The findings in patients with low baseline hematocrit may be compatible with speculation that the association between hematocrit and morbidity/mortality is non-linear, perhaps J- or U-shaped. However, cautious interpretation is needed because end-stage renal disease patients are not at low mortality risk.

ASSOCIATION BETWEEN ELEVATED HEMATOCRIT AND T2DM

A further factor potentially linking hematocrit with mortality is its association with insulin resistance and impaired insulin secretion. This is important because adult-onset TD is associated with T2DM with greater use of TTh in these patients and risk of further increased hematocrit levels. It is, therefore, perhaps

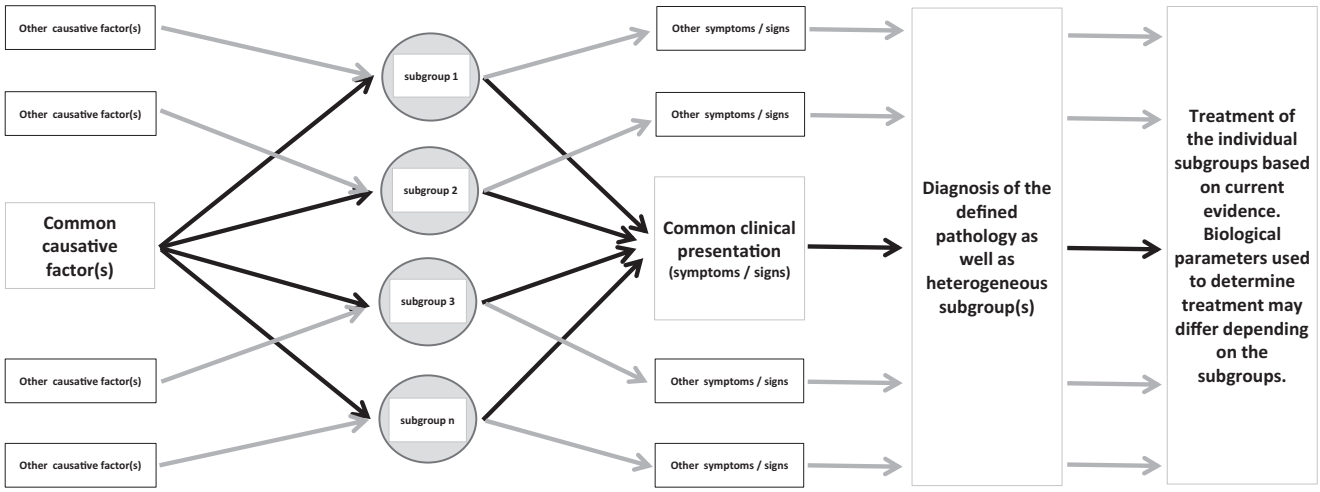

Figure 2. A diagrammatic scheme of the importance of heterogeneity in complex diseases. From: Ramachandran S, König CS, Hackett G, Livingston M, Strange RC. Managing clinical heterogeneity: An argument for benefit based action limits. *Journal of Medical Diagnostics and Therapy* 2018;1:034701 (permission to use the figure was obtained from the journal).

reasonable to consider T2DM patients as a subgroup when studying the clinical consequence of elevated hematocrit. Facchini et al[39] reported that increases in hematocrit and hemoglobin levels are associated with increased insulin resistance (by measuring steady-state plasma glucose levels after a 180-minute infusion of somatostatin, insulin, and glucose), compensatory hyperinsulinemia, elevated blood pressure, triglycerides, and lower high-density lipoprotein cholesterol values in 150 individuals. When adjusted for all of the above factors in a multiple regression analysis, only insulin resistance and plasma insulin response to oral glucose remained associated with hematocrit and hemoglobin levels. In a prospective study of 7,193 middle-aged men, Wannamethee et al[40] found an independent association between hematocrit and development of T2DM, independent of age, BMI, smoking, physical activity, high-density lipoprotein cholesterol, and systolic blood pressure. T2DM was significantly higher in men with hematocrit levels $\geq$ 0.48, compared with levels <0.42 (RR = 4.5; 95% CI = 2.5−6.3, adjusted for age and BMI). Even after further adjustment for predictors of T2DM with which hematocrit is correlated, there remained a linear association with the risk of T2DM. The authors recommended that hematocrit, which is a major determinant of whole blood viscosity, should be added to the cluster of risk factors that link T2DM with CVD. As previously stated in the BLAST randomized controlled study HbA1c levels were not associated with baseline hematocrit levels.

Clearly, monitoring hematocrit levels in men on TTh is essential.[4] A hematocrit level of 0.54 has been accepted as the level at which down-titration/discontinuation of TTh is recommended. Although studies hint at an association between hematocrit, CVD, and all-cause mortality, there is no consensus view.

It is reasonable to speculate that increased hematocrit levels result in altered blood flow characteristics that may lead to increased morbidity/mortality. Thus, it would be useful to characterize the blood flow changes that occur at different hematocrit levels in arteries varying in diameter. It is important that heterogeneity of disease pathogenesis is recognized since the adverse effects of TTh may only be seen in certain patient subgroups (Figure 2).[25] Subgroups may be identified by broad phenotypes, including age, diabetes, and previous CVD, as well as by those specific to adult-onset TD and TTh: baseline testosterone and hematocrit, sexual health symptoms, and other clinical consequences of hypotestosteronemia. Subgroups may also be based on the extent of response to TTh or use of concomitant treatments such as antihypertensives, statins, and phosphodiesterase 5-inhibitors. In this way, the appropriateness of using a hematocrit level of 0.54 as the sole cutoff for clinical intervention can be assessed.

EFFECTS OF HEMATOCRIT ON BLOOD FLOW CHARACTERISTICS

A change in hematocrit is likely to affect blood flow, due to increased viscosity leading to altered endothelial function and perfusion. This may, in part, explain the negative outcomes reported in some studies of TTh. We now focus on some theoretical considerations of the effects of hematocrit on blood flow. Blood viscosity is a key factor determining blood flow. Blood is a multicomponent fluid comprising cellular elements (platelets, leucocytes, erythrocytes) and plasma. Blood behaves as a non-Newtonian fluid, exhibiting shear-thinning behavior, as well as being viscoelastic and thixotropic.[41] Although plasma alone was considered a Newtonian fluid for decades, more recent studies have shown that blood plasma has a noticeable viscoelastic behavior.[42,43] Erythrocytes, which are deformable, constitute the majority of the suspended elements in blood and

have a propensity to aggregate at low shear rates, forming rouleaux.

As with most particle suspensions the viscosity of whole blood depends primarily on hematocrit, erythrocyte aggregation, and deformability.[44] Blood viscosity is reported to increase exponentially[45] or quadratically[46] with hematocrit, with the effect of hematocrit being more pronounced at lower shear rates because of its effect on erythrocyte aggregation contributing further to the shear thinning behavior.[47] Wall shear is known to be associated with activating endothelial function. Piety et al[48] found that increasing the hematocrit of erythrocyte suspensions in plasma in vitro from 20−60% resulted in a 3.5-fold increase in viscosity at a shear rate of 129 s^{-1} and a 17.5-fold increase at 0.3 s^{-1}. Empirical correlations and various concentration dependent models have been developed to describe this effect of hematocrit on blood viscosity.[46,49,50] These will allow for improved of calculated wall shear stress in both micro- and macrocirculation.

It is well established that the vascular bed affects hematocrit distribution, with hematocrit levels being lower in the microvasculature. When blood flows from a large vessel to a small-diameter one (≤0.3 mm), the observed hematocrit level decrease is known as the Fåhræus effect.[51] The complex bifurcating microvascular architecture in conjunction with the particulate nature of blood gives rise to phenomena such as erythrocyte migration away from the wall and plasma skimming, resulting in higher flow rate branches receiving more cells and blood with a higher hematocrit.[52] This results in highly heterogeneous hematocrit distributions in the microcirculation that cause local variations in viscosity and flow resistance. This has been demonstrated in vivo[53] and in recent microfluidic studies of blood flow.[54,55]

In addition to the Fåhræus effect, the particulate nature of blood is also responsible for phenomena such as leucocyte and platelet margination,[56] where these smaller cells are observed to migrate toward the vessel wall. Increased hematocrit could lead to more interactions between erythrocytes and leucocytes or platelets promoting margination. Interestingly, Walton et al[57] recently showed that elevated hematocrit in mice promoted arterial thrombosis, perhaps due to rapid platelet accumulation within the thrombus.

Aging and, importantly, T2DM in the context of TTh can impair blood fluidity, altering tissue perfusion, perhaps leading to functional deterioration. It is well established that blood fluidity becomes impaired with age and erythrocyte life span.[58,59] Disorders such as sickle cell disease[60−62] and diabetes[63−67] have been associated with reduced erythrocyte deformability.

Lower deformability of erythrocytes in T2DM has been associated with poor glycemic control and microvascular complications, such as diabetic retinopathy.[68] Erythrocytes in individuals with T2DM also undergo morphologic changes, with their shape deviating from the established biconcave disc to a more elongated shape.[69] These changes together with enhanced erythrocyte aggregation in T2DM can result in elevated blood viscosity, which may be a factor in the pathogenesis of microvascular disease and non-flow limiting coronary artery disease.[70,71] It is possible this risk is exacerbated by TTh-associated increase in hematocrit.

Changes in macrovascular flow characteristics that may be associated with increased hematocrit are also potentially important. Both end-diastolic and peak systolic velocities have been associated with atherogenesis.[72−76] Our research group found that lower peak systolic velocity, based on ultrasound measurements in the carotid artery, was associated with coronary heart disease.[72]

OPTIMAL HEMATOCRIT

Salazar-Vazquez et al[77] suggested that the treatment of diabetes should target the maintenance of an optimal hematocrit to lower cardiovascular risk, prompting the question what is the ideal hematocrit? Clinical guidelines use a value <0.54 based on a population distribution and not physiological evaluation. Increased hematocrit should theoretically increase tissue oxygenation, because oxygen content varies linearly with hematocrit. However, it also increases blood viscosity in an exponential function reducing blood flow; hence, an ideal hematocrit should exist that optimizes tissue oxygenation and flow performance. A recent in vitro study concluded that the optimum hematocrit is different for large and small vessels, attributed to the difference in driving pressures and, hence, perfusion rates.[48] Many cardiovascular conditions result in lower blood flow rates, either in systemic circulation or locally, which might result in the optimal hematocrit being lower than the physiological. This could be due to rheological changes brought about by impaired erythrocyte deformability or increased erythrocyte aggregation, endothelial dysfunction, or decreased cardiac output.[45] An increase in hematocrit will increase whole blood viscosity, which, in turn, will require a high blood pressure to maintain flow. Although the required increase in blood pressure can be quantified easily in vitro, the situation is more complex in vivo. Factors such as altered vessel elasticity, endothelial function, and release of vasodilators (eg, nitric oxide) may influence the compensatory mechanisms. The above makes a case for individuals with T2DM to be considered as a subgroup, because the efficiency of these factors may differ from that in health. In sickle cell anemia, for example, the optimum hematocrit for transfusion has been set <0.30.[78] This implies that the optimum hematocrit in disease is subgroup specific and depends on the many factors impacting blood rheology.

Therapeutic phlebotomy is the mainstay of controlling hematocrit in polycythemia vera. Some experts suggest hematocrit targets <0.45% in men and <0.42% in women.[79−82] A prospective trial randomly assigned 365 adults with polycythemia vera to more-intensive treatment (target hematocrit < 0.45) vs less-intensive treatment (target hematocrit = 0.45−0.50%); control of hematocrit achieved by phlebotomy, hydroxyurea, or

both. After a median follow-up of 31 months, compared with the more-intensive therapy group, the less-intensive therapy was associated with shorter time to death from CVD or major thrombotic events (HR = 3.9, 95% CI = 1.5−10.5]; with events reported in 10% of the less-intensive therapy group and 3% in the more-intensive treatment group.[80]

CONCLUSION

Although TTh use has increased, with most studies demonstrating benefit, doubts of its safety based on a few controversial reports of increased CVD remain. We have seen that increased hematocrit is the most common adverse effect of TTh, and guidelines regarding action thresholds are based on a population-derived level of 0.54. Longitudinal studies suggest that hematocrit influences CVD morbidity and mortality, although the association may not be linear. It is clear that further studies are required, and we propose that, in addition to clinical studies with hard and surrogate endpoints, changes in blood flow characteristics should be evaluated across macro- and microcirculatory vascular beds.

Advanced computational tools are required to understand the particulate nature of blood in the microcirculation, taking into account the impact of increased hematocrit and altered erythrocyte properties. Although this has been carried out in some pathologies (sickle cell anemia,[83] malaria[84]), only simple vascular geometries rather than networks were considered. Microfluidics has allowed microscale blood flow characteristics to be probed, allowing cell and flow distribution to be resolved and phenomena such as erythrocyte aggregation and deformability on those to be studied in detail.[52,53] Thus, concurrent studies of clinical outcomes and evaluation of flow changes after hematocrit change during TTh in different patient groups will allow management guidance based on evidence that allows for patient heterogeneity.

Corresponding Author: Professor S. Ramachandran, Department of Clinical Biochemistry, University Hospitals Birmingham NHS Foundation Trust, Good Hope Hospital, Rectory Road, Sutton Coldfield, West Midlands B75 7RR, United Kingdom. Tel: +44-121-424 7246; Fax: +44-121-311 1800; E-mail: sud.ramachandran@heartofengland.nhs.uk

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STATEMENT OF AUTHORSHIP

Category 1

a) **Conception and Design**
Carola S. König; Stavroula Balabani; Geoffrey I. Hackett; Richard C. Strange; Sudarshan Ramachandran

b) **Acquisition of Data**
Carola S. König; Stavroula Balabani; Geoffrey I. Hackett; Richard C. Strange; Sudarshan Ramachandran

c) **Analysis and Interpretation of Data**
Geoffrey I. Hackett; Sudarshan Ramachandran

Category 2

a) **Drafting the Article**
Carola S. König; Stavroula Balabani; Geoffrey I. Hackett; Richard C. Strange; Sudarshan Ramachandran

b) **Revising it Critically for Important Intellectual Content**
Carola S. König; Stavroula Balabani; Geoffrey I. Hackett; Richard C. Strange; Sudarshan Ramachandran

Category 3

a) **Final Approval of The Version to be Published**
Carola S. König; Stavroula Balabani; Geoffrey I. Hackett; Richard C. Strange; Sudarshan Ramachandran

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