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## THROMBOELASTOGRAPHY®

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### 6.1 CHAPTER OBJECTIVE

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The objective that directed the research in this chapter was:

- To analyse the viscoelastic properties of WB during clot formation in BCa patients compared to the WB of healthy individuals using TEG®

### 6.2 INTRODUCTION

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Thromboelastography® is a viscoelastic test (VET) that was established in 1948 by Hartert<sup>298</sup> and is a technique used for the rapid analysis of the rate and strength of clot formation and WB clot lysis.<sup>299</sup> Some researchers favour this technique above traditional coagulation tests when analysing the hypercoagulable state as well as haemorrhage risk of prostate cancer patients, haemophilia, postpartum haemorrhage, and patients undergoing cardiac or brain trauma surgery.<sup>300-303</sup>

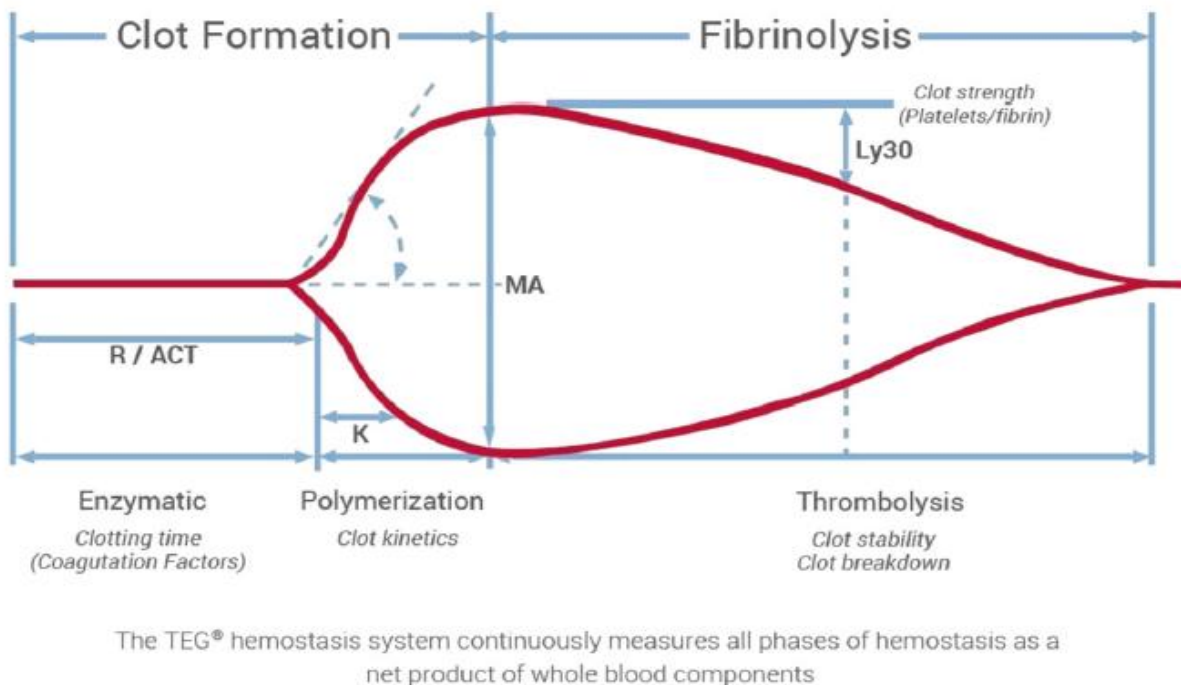
TEG® is primarily sensitive to three blood components: levels of fibrinogen in WB and plasma, Hct, and MP levels in plasma.<sup>304</sup> This technique proves specifically useful since it continuously measures clot formation, clot strength, PLT function and clot lysis whereas other coagulation tests typically only provide readings until the phase of first fibrin strand formation.<sup>305</sup>

A TEG® activates the coagulation system of a small sample of blood *ex vivo* and monitors active changes of clot formation and lysis.<sup>306</sup> By inducing coagulation, TEG® analysis records the viscoelastic changes that take place during coagulation.<sup>298</sup> The parameters measure viscoelastic changes in WB without separating plasma.<sup>5</sup> Thromboelastographs can be used to identify hyper- or hypocoagulation as well as the severity of these coagulopathies using WB and PLT poor plasma.<sup>261, 299, 307-312</sup>

This technique may also serve as an indirect measure of thrombin generation (TG).<sup>308</sup> Tripodi et al. found that the WB TEG® profiles of  $\beta$ -thalassemia patients showed hypercoagulability but not in the PLT-poor plasma TG profiles, thereby suggesting blood cell components and/or PLTs to be the primary determinants of thrombotic risk.<sup>313</sup>

A typical presentation of TEG<sup>®</sup> results is shown in Figure 13. The data is presented in a graph with time on the horizontal axis in minutes, and clot firmness on the vertical axis. There are several factors of coagulation analysed by the TEG<sup>®</sup>. The maximum amplitude (MA) is measured in millimetres and measures the overall clot stiffness and is used to evaluate primary and secondary haemostasis.<sup>299</sup> It is also useful in guiding PLT transfusion.<sup>314</sup> An MA of < 50-55 mm indicates ineffective PLT involvement in coagulation which signals PLT transfusion. A lower MA specifies lower PLT numbers as well as PLT dysfunction.<sup>299</sup>

## Measure all phases of hemostasis in whole blood.



**Figure 13:** A typical presentation of a thromboelastographic tracing curve. Clot formation and lysis are illustrated by the respective expansion and contraction of the curve.<sup>5</sup>

The R-time is a measure of the time it takes from the start of measurement to the start of clot formation. A higher R time is associated with lower concentration of clotting factors which indicates the need for factor replacement.<sup>299</sup> The k-time indicates the time taken from the start of clot formation until the clot firmness reaches an amplitude 20 mm. An  $\alpha$ -angle of < 45° indicates low of dysfunctional fibrinogen which calls for fibrinogen replacement.<sup>299</sup> It is important to keep in mind that overall coagulation is different between males and females due to the differences in haematocrit and plasma content in the respective sexes.<sup>304</sup>

Furthermore, secretion of sex-specific growth hormones (particularly oestrogen) mediate differences in thrombosis due to changing the expression of coagulation inhibitor genes found in the liver.<sup>315, 316</sup> The outcome hereof being that men are more susceptible to thrombosis than women.<sup>315</sup>

### 6.3 MATERIALS AND METHODS

#### Thromboelastography® for the quantitative analysis of the viscoelastic properties of whole blood

Thromboelastography® is sensitive to the time from venepuncture to analysis. Citrated blood can be safely analysed within 8 hours of drawing the blood.<sup>317</sup> The TEG® assays were conducted using a TEG® 5000 computer-controlled device (Haemoscope Corp., Niles, IL, USA) and by using WB. The TEG® assays were conducted as indicated by manufacturer-guidelines using TEG® kits. The standard TEG® reaction should have 340 µL of WB from a sodium citrate tube. To a loaded TEG® cup, 20 µL of 0.2 M calcium chloride was added. After adding the calcium chloride, 340 µL of WB was then added and the assay was left to run. All the reagents used for TEG® during this study are listed and detailed in Appendix G.

Coagulation and clot kinetics were then monitored by the specialised TEG® computer program. This was measured based on pre-programmed parameters. Coagulation ended once the parameters were met, and the results were reported both numerically and as a graphical tracing. As only clot formation is relevant to this study, the test was terminated once MA was reached. Table 10 presents a summary of different effects on coagulation depending on the increase or decrease of each TEG® parameter. Table 11 explains the standard TEG® tracing and viscoelastic parameters that were used in this study.

**Table 10:** Summary of the viscoelastic parameters, their units and descriptions that was studied using TEG®. Adapted from Pretorius et al.<sup>261</sup>

Code	Parameter	Reference ranges <sup>305, 318-320</sup>	Units	Description
R	Reaction time	9-27	Minutes	Rate of initial clot formation with an amplitude of 2 mm.

<b>α</b>	Angle	2-58	Degrees	Rate by which clot amplitude increases (i.e., rate of clot formation). Reflects the speed of fibrin build up and cross linking. Represents the slope between the representative traces by R and K.
<b>K</b>	Clot kinetics	2-9	Minutes	The time it takes from initial clot formation to a clot reaching an amplitude of 20 mm. Reflects the speed of clot polymerisation
<b>MA</b>	Maximum amplitude	44-64	mm	The maximum stiffness of the developed clot. Reflects fibrin clot strength (i.e., overall clot stability).
<b>G</b>	Shear elastic modulus strength	3.6-8.9	Dynes/cm <sup>2</sup>	Resistance of clot to deformation
<b>TMRTGG</b>	Time to the maximum rate of thrombus generation	5-23	Minutes	Time interval before maximum speed of clot growth
<b>MRTGG</b>	Maximum rate of thrombus generation	0-10	dynes/cm <sup>2</sup> s	The maximum velocity of clot growth or maximum rate of thrombus generation using G.
<b>TGG</b>	Total thrombus generation	251-1014	Dynes/cm <sup>2</sup>	Total clot strength. Indicates the amount of total resistance generated during clot formation. Total area under the velocity curve during growth of the clot, representing the amount of clot strength generated during clot growth.

**Table 11:** Interpreting TEG<sup>®</sup> results in terms of coagulopathy.<sup>261</sup>

Parameter	Hypocoagulable		Hypercoagulable	
<b>R</b>	↑	Clots form slower	↓	Clots form faster
<b>K</b>	↑	Clot reaches 20 mm strength slower	↓	Clot reaches 20 mm strength quicker
<b>A</b>	↓	Decreased thrombin burst resulting in less fibrin fibre crosslinking	↑	Increased thrombin burst resulting in more fibrin fibre crosslinking
<b>MA</b>	↓	Decreased PLT and/or fibrin interaction which results in less dense, less rigid clot	↑	Increased PLT and/or fibrin interaction which results in a more dense, more rigid clot
<b>MRTGG</b>	↓	Decreased clot growth	↑	Increased clot growth
<b>TMRTGG</b>	↑	Increased time from the initiation of clot formation to maximum clot formation	↓	Decreased time from initiation of clot formation to maximum clot formation
<b>TGG</b>	↓	Decreased clot strength	↑	Increased clot strength

## 6.4 STATISTICAL ANALYSIS

All participants' TEG<sup>®</sup> parameters that were measured are presented in Table 12 and 13 for the control and patient groups respectively. The results from the statistical

analysis are summarised in Table 14. The normality test, Shapiro-Wilk test, was performed. For non-parametric data the Mann-Whitney Wilcoxon two-sided statistical test was performed for the analysis of all eight parameters. The data is presented as a median with minimum and maximum values.

## 6.5 RESULTS

The results obtained from the TEG<sup>®</sup> analyses for the control and patient groups are tabulated in Tables 12 and 13 respectively. These results show the TEG<sup>®</sup> parameters that make up the coagulation profiles of each individual.

**Table 12:** TEG<sup>®</sup> raw data obtained from the TEG<sup>®</sup> analyses of the control group.

Control	TEG <sup>®</sup> results							
	R	K	A	MA	G	MRTGG	TMRTGG	TGG
C1	13,80	3,30	55,90	67,50	10,40	4,24	21,17	1061,26
C2	13,30	4,20	50,70	66,50	9,90	3,99	19,08	1011,00
C3	9,60	5,20	49,60	49,50	4,90	2,39	15,58	497,76
C4	9,80	4,80	52,20	65,00	9,30	2,94	19,33	944,76
C5	10,40	6,80	45,00	55,00	6,10	1,82	19,50	622,15
C6	11,20	4,20	51,40	68,50	10,90	4,58	17,67	1109,86
C7	9,00	2,80	61,90	65,50	9,50	4,24	14,00	958,54
C8	8,30	3,10	60,20	54,50	6,00	3,62	13,33	598,74
C9	9,90	4,90	50,80	55,50	6,20	2,43	15,42	626,48
C10	8,50	3,20	57,90	67,50	10,40	4,41	14,58	1048,67
C11	8,90	3,10	59,90	57,00	6,60	5,42	12,08	664,52
C12	13,20	2,30	57,90	61,60	8,00	6,15	16,08	802,89
C13	9,80	2,60	54,00	64,60	9,10	6,84	13,75	934,31
C14	11,80	3,70	43,80	59,40	7,30	4,14	16,17	734,62
C15	16,20	5,70	35,60	58,70	7,10	3,07	23,42	717,05
C16	12,00	2,40	58,20	62,70	8,40	5,69	15,17	844,27
C17	5,30	1,60	64,70	63,40	8,60	7,69	7,33	867,8
C18	9,90	3,20	47,30	52,20	5,50	3,87	12,83	552,9
C19	13,20	4,10	41,70	61,00	7,80	3,33	19,42	786,58
C20	11,80	3,70	25,70	50,10	5,00	3,50	14,75	503,01
C21	7,20	3,30	33,30	56,80	6,60	3,58	9,08	660,15
C22	11,40	4,80	50,80	69,00	11,10	2,52	15,42	1135,40
C23	7,90	6,10	40,50	63,50	8,70	4,44	17,92	884,71
C24	6,10	2,80	63,40	51,50	5,30	3,75	9,83	533,35

<b>C25</b>	11,80	3,60	54,50	59,50	7,40	4,32	16,58	732,93
<b>C26</b>	21,50	7,50	40,00	45,50	4,20	1,88	29,67	422,86
<b>C27</b>	15,00	5,80	42,70	63,10	8,50	3,91	23,80	871,26
<b>C28</b>	12,20	3,60	55,60	62,00	8,20	4,65	16,33	825,28
<b>C29</b>	14,10	2,80	61,50	62,00	8,20	5,50	17,50	821,39
<b>C30</b>	18,20	5,80	43,20	59,00	7,20	3,33	27,08	727,44
<b>C31</b>	23,30	7,00	48,10	50,50	5,10	2,19	26,75	512,76
<b>C32</b>	13,90	3,00	60,80	54,00	5,90	4,33	17,58	596,02
<b>C33</b>	16,60	9,20	40,00	46,00	4,30	1,49	27,58	433,31
<b>C34</b>	11,60	2,40	66,30	68,00	10,60	13,01	14,50	1060,80
<b>C35</b>	8,80	2,20	71,20	73,30	13,70	11,38	12,70	1254,6

R: reaction time; K: kinetics; A: angle; MA: maximum amplitude; G: shear elastic modulus strength; MRTGG: maximum rate of thrombus generation; TMRTGG: time to maximum rate of thrombus generation; TGG: total thrombus generation.

**Table 13:** TEG<sup>®</sup> raw data obtained from the TEG<sup>®</sup> analyses of the patient group.

Patient	TEG <sup>®</sup> results							
	R	K	A	MA	G	MRTGG	TMRTGG	TGG
<b>P01</b>	5,00	1,40	53,50	70,20	11,80	9,51	7,08	1183,91
<b>P02</b>	8,20	2,80	53,80	51,60	5,30	4,09	10,33	535,68
<b>P03</b>	6,60	1,80	48,40	70,70	12,10	8,08	9,92	1215,70
<b>P04</b>	4,70	1,60	68,50	73,60	13,90	10,53	7,67	1402,19
<b>P05</b>	7,80	1,60	66,70	60,70	7,70	7,28	9,25	773,10
<b>P06</b>	9,70	2,20	54,20	65,00	9,30	6,32	12,5	931,00
<b>P07</b>	10,30	2,80	49,00	57,90	6,90	5,88	18,08	690,90
<b>P08</b>	5,80	1,90	62,00	58,20	6,90	5,64	7,08	702,90
<b>P09</b>	3,30	8,80	39,70	29,30	2,10	1,87	3,92	206,93
<b>P11</b>	20,10	5,80	40,40	74,10	14,30	3,68	24,00	912,63
<b>P12</b>	11,10	2,60	53,30	66,60	10,00	6,59	15,33	1001,88
<b>P13</b>	15,80	5,80	33,80	61,20	7,90	2,97	24,17	792,97
<b>P14</b>	19,90	5,20	38,00	59,00	7,20	3,48	28,25	721,24
<b>P15</b>	13,20	7,20	28,60	60,00	7,50	2,68	21,50	752,76
<b>P16</b>	14,20	3,20	50,80	60,90	7,80	5,33	18,83	779,28

<b>P17</b>	7,70	2,10	61,80	72,50	13,20	7,32	11,58	1325,06
<b>P18</b>	6,80	1,60	57,90	68,00	10,60	8,59	9,33	1066,80
<b>P19</b>	7,80	1,90	62,80	70,40	11,90	7,81	6,70	1191,60
<b>P20</b>	5,80	2,60	41,30	68,70	11,00	5,21	9,83	1106,10
<b>P21</b>	3,20	1,20	72,80	67,50	-	9,16	4,83	1045,80
<b>P22</b>	13,20	3,90	47,90	67,30	10,30	6,46	20,25	1033,10
<b>P23</b>	8,80	4,20	36,20	67,80	-	2,56	24,00	623,01
<b>P24</b>	15,60	6,00	30,40	55,40	-	4,09	16,08	1059,77
<b>P25</b>	12,00	5,70	32,10	52,60	-	2,31	17,92	557,35

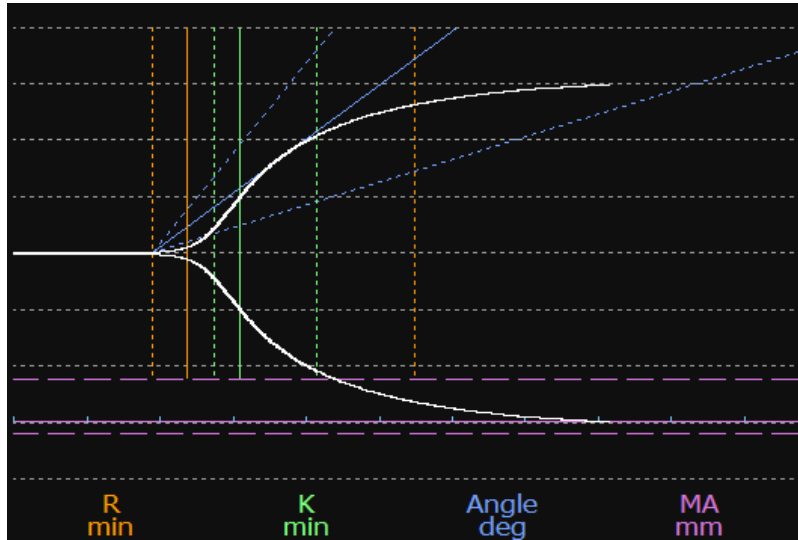
R: reaction time; K: kinetics; A: angle; MA: maximum amplitude; G: shear elastic modulus strength; MRTGG: maximum rate of thrombus generation; TMRTGG: time to maximum rate of thrombus generation; TGG: total thrombus generation.

**Table 14:** The TEG<sup>®</sup> data comparing the summary statistics of the control and patient groups. The median, minimum value, maximum value, as well as P-value is showed. A p-value of  $\leq 0.05$  indicates significance.

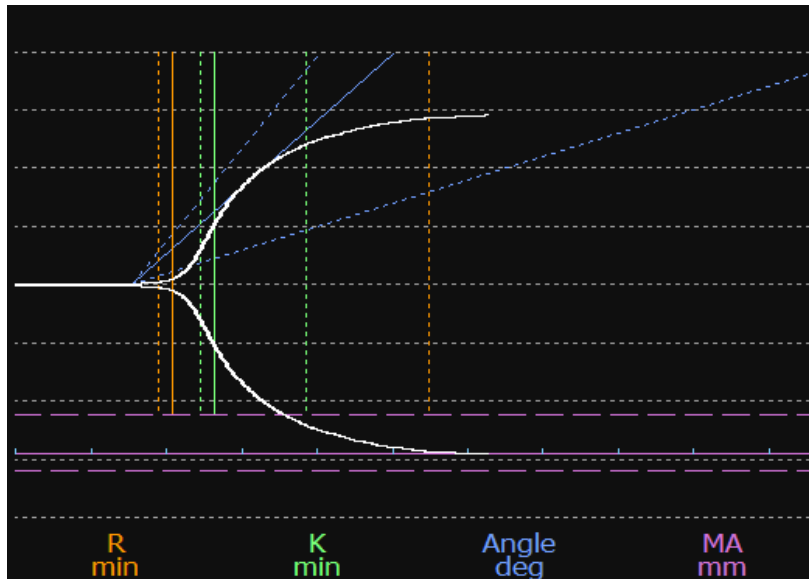
Parameter	Median (min; max)		P-value
	Control group n = 35	Patient group n =25	
<b>R</b>	11.60 (5.30;23.30)	8.00 (3.20;20.10)	0.01 *
<b>K</b>	3.60 (1.60;9.20)	2.60 (1.20;8.80)	0.02 *
<b>Ang</b>	51.40 (25.70;71.20)	49.90 (28.60;72.80)	0.58
<b>MA</b>	61.00 (45.50;73.30)	66.10 (29.30;74.10)	0.02 *
<b>G</b>	7.80 (4.20;13.70)	9.50 (2.10;14.30)	0.03 *
<b>MRTGG</b>	3.99 (1.49;13.01)	5.86 (1.87;10.53)	0.01 *
<b>TMRTGG</b>	16.17 (7.33;29.67)	10.96 (3.92;28.25)	0.01 *
<b>TGG</b>	786.60 (422.90;1255.00)	945.10 (206.90;1402.00)	0.02 *

R: reaction time; K: kinetics; A: angle; MA: maximum amplitude; G: shear elastic modulus strength; MRTGG: maximum rate of thrombus generation; TMRTGG: time to maximum rate of thrombus generation; TGG: total thrombus generation; \*: significant difference

Figures 14 and 15 show representative graphs of the control and patient groups that showcased the most representative TEG<sup>®</sup> runs of the respective groups. The graphs of the patient group showed more variation in the TEG<sup>®</sup> values than that of the control group.



**Figure 14:** Representative graph of the control group TEG<sup>®</sup>. Showing all parameters within the reference ranges. R: reaction time; K: kinetics; MA: maximum amplitude.



**Figure 15:** Representative graph of the patient group TEG<sup>®</sup>. Showing faster R and K times, normal angle, and increased MA. R: reaction time; K: kinetics; MA: maximum amplitude.

## 6.6 DISCUSSION

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This method was chosen in the study to quantify the viscoelastic properties of clot formation in primary BCa patients. In a hypercoagulable state, there is a typical profile seen such as an increase in  $\alpha$ -angle, MA, MRTGG, and TGG as well as a decrease in the R-time, K-time, and TMRTGG.<sup>261</sup> In the recorded TEG<sup>®</sup> results, there was no significant difference between the  $\alpha$ -angle measurements of the control and patient groups. However, MA, G, MRTGG, and TGG were increased in the patient group. Additionally, R-time, K-time, and TMRTGG were decreased in the patient group. When compared to healthy reference ranges obtained from literature, most of the patient group values were still within the healthy reference ranges even though it was different from the control group values obtained in this study, indicating rather a tendency to hypercoagulate. Only the significant differences will be discussed here by using the summarised information presented in Tables 10 and 11 for the interpretation of the results.

The observed decreased R-time is indicative of faster clot formation which aligns with hypercoagulability in the patient group. An increased G value is also indicative of a hypercoagulability since the clots formed in the patient group were more rigid and therefore more resistant to change. A decreased K measurement indicates that the clot strength is reached quicker and further corresponds to hypercoagulability in the patient group. Since the MA is higher in the patient group than in the control group, it can be concluded that there is increased fibrin and/or PLT interaction in the patient group which results in more dense and rigid clots.<sup>261</sup> This too is consistent with hypercoagulability. This increased MA is confirmed by the SEM micrographs discussed in Chapter 5 that show increased fibre interaction, tangling, and denser areas of fibres when compared to the control group micrographs.

The MRTGG is a measure of the maximum velocity of clot growth (or maximum rate of thrombus generation), Since the MRTGG was higher in the patient group, it can be concluded that the clot growth is increased in the patient group, indicating hypercoagulability. Since TMRTGG represents the time before the maximum rate of clot growth, a decreased TMRTGG value as observed in the patient group, suggests that the maximum rate of clot growth was reached faster in the patient group. This decreased value

represents a hypercoagulable parameter. The TGG measurement is an indication of the amount of total resistance generated during clot formation, i.e., total clot strength. The TGG being higher in the patient group therefore confirms that this parameter is also consistent with hypercoagulability.

Panigada et al. used TEG<sup>®</sup> to investigate the effects of COVID-19 on the haemostasis of infected patients. The results showed that there was a significant increase in the hypercoagulability of patients that tested positive for COVID-19.<sup>309</sup> The TEG<sup>®</sup> results showed a specific decrease in the K values and increase in the MA values which was indicative of a faster clotting time as well as fibrin clot strength respectively.<sup>309</sup> This study suggests a possible reason for the hypercoagulability may be an increase in circulating MVs. These particles may stem from monocytes and PLTs that are carriers of procoagulants.<sup>309</sup> This is a plausible explanation because circulating MVs may trigger a hypercoagulable state, and are shown to be associated with increased VTE risk.<sup>321</sup>

Using the results of TEGs<sup>®</sup> on the WB of prostate cancer patients, Toukh et al. found evidence of hypercoagulability in this patient group. This finding was most significant in metastatic cancer patients. The parameters most affected by this type of cancer was the R-time, which was significantly reduced and the angle, and MA which showed significant increases when compared to the controls in this study.<sup>322</sup> The researchers also highlighted the TF expressing MVs and their role in coagulation initiation.<sup>322</sup>

The increase in MA can be supported by the changes in PLT interaction with the fibrin fibres also observed with SEM. The denser appearance of the fibres would allow for more interaction and a closer association between the fibres, resulting in a denser clot as reflected by the increased MA values. Since there was also increased PLT presence and interaction observed in the micrographs of the SEM, this further supports the likelihood that the PLTs might be more active in BCa due to the increased inflammatory state, and then interacts with fibrin, resulting in stronger, more rigid clots.<sup>323</sup> The decrease in K time shows that these clots are not only stronger, but also forms quite quickly. The increase in MRTGG may be due to the increased inflammatory state resulting in activation of the coagulation cascade which causes TF and FXII activation, resulting in an increase in thrombin

generation.<sup>155, 324</sup> This increase results in the increased MRTGG observed and contributes to the clots forming faster than in the control group.

Thrombotic events in cancer patients, specifically those that include denser, stiffer clots that are more resistant to lysis are associated with increased inflammation and oxidative stress, and mainly fibrin-related mechanisms.<sup>325</sup> This is due to cancer cells producing and releasing inflammatory cytokines (IL-6), prothrombic MPs, procoagulant and fibrinolytic proteins (MMPs, VEGF), all contributing to hypercoagulability which can result in thrombotic events.<sup>326</sup>

## 6.7 CONCLUSION

Based on the results obtained from the TEG<sup>®</sup>, it is evident that there is hypercoagulability present in the patient group based on seven out of the eight measured parameters matching a hypercoagulable profile. Though not all the samples of the patient group held to a hypercoagulable profile, the group as a whole did present as hypercoagulable. It thereby confirms that clot formation in these patients is changed and could hold a potential risk for the development of clotting complications in these patients. If the BCa status is combined with treatment or other procoagulant co-morbidities it could only elevate the risk to thrombosis in these patients. The increase in MA could possibly be a result of pro-clotting factors that are activated because of the increased inflammatory state. The pro-clotting factor may be fibrinogen as there was also tightly woven fibres seen in Chapter 5. Therefore, future studies could include the measurement of fibrinogen to determine if fibrinogen may be the pro-clotting factor that is responsible for the denser, faster forming clots observed through the decreased K values, increased MA values, as well as the SEM images discussed in Chapter 5.

# CHAPTER SEVEN

## CONCLUSION AND FUTURE DIRECTION

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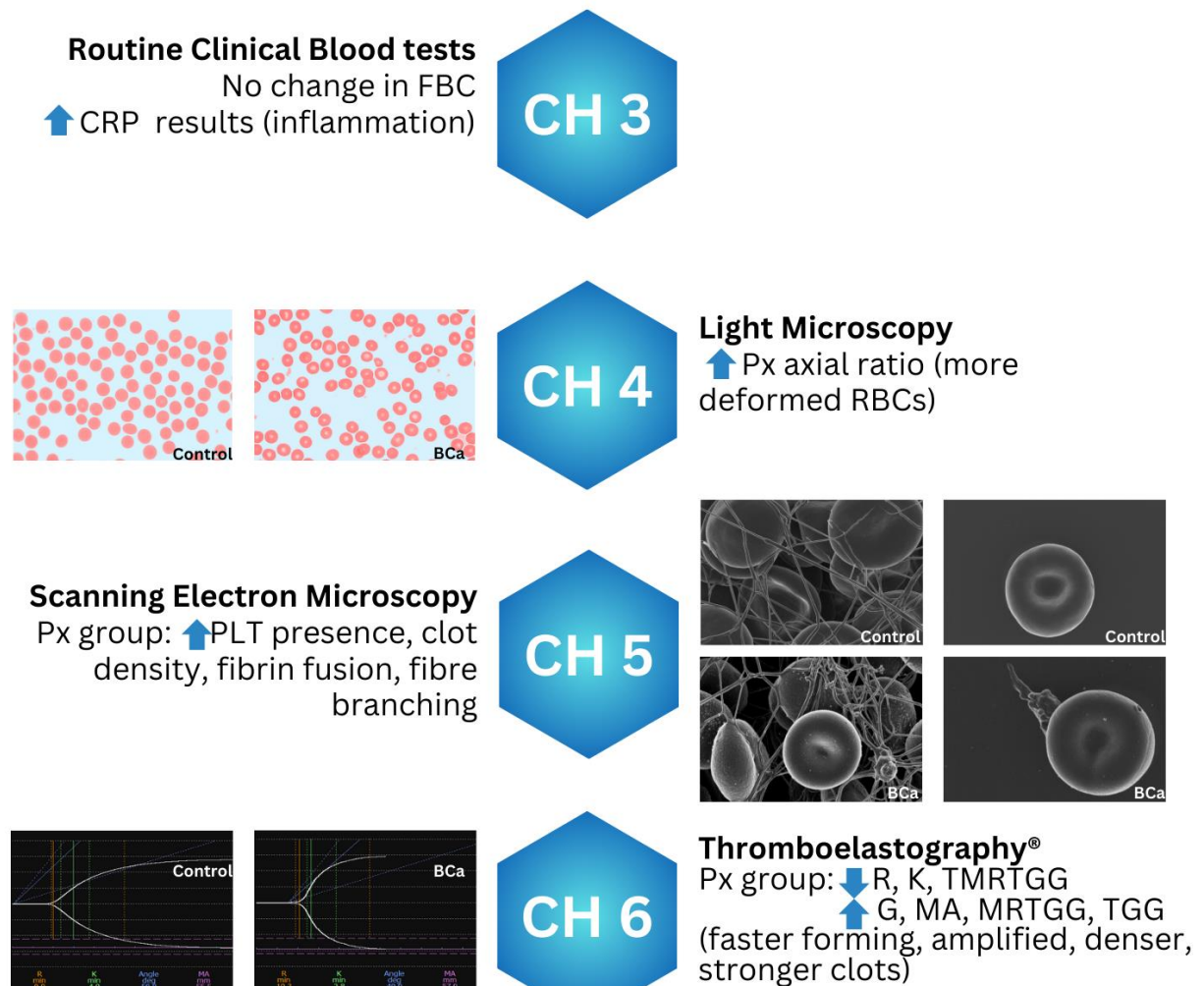
### DISCUSSION AND CONCLUSION

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Cancer has been and remains one of the most studied diseases of life. Specifically, BCa, which has maintained the attention of researchers all over the world. Brain cancer is known for having a generally poor prognosis and a high mortality rate. There is also a known association between cancer and thrombotic events. This poses a dilemma that still lacks a sure solution. To reduce and manage the number of thrombotic events that occur in cancer patients, there must be more research dedicated to informing therapeutic strategies that treats the underlying cause. Research on metastatic BCa is on the rise, however, to assess the risk that patients with primary, non-metastatic BCa face is not widely researched. Therefore, this study was aimed at investigating the haematological and clotting properties of these patients to investigate and identify the prominent factors that could contribute to the potential risk for hypercoagulability associated with primary, non-metastatic BCa. Hypercoagulability poses a serious risk for contributing to PE and/or DVT development in the lower limbs of patients<sup>309</sup> and should therefore be taken into consideration when treatment is established for this patient group. Figure 16 summarises the main findings of each of the research chapters (Chapters 3 - 6).

The combined chapters in this dissertation showcase an overall biophysical coagulation profile in a population of BCa patients. This was then compared to healthy controls and a number of alterations were identified in the patient group. In Chapter 4, the average axial ratio measurements of the patient group were significantly higher than that of the control group. This established that the patient group had more deformed RBCs present per sample taken compared to the control group. When the shapes of RBCs change, there is a potential risk created due to its possible impact on the viscosity and sheer stress of blood<sup>327</sup> moving especially within the smaller venules, arterioles, and capillaries.

The most prominent findings from the SEM analyses were the altered fibres within the WB clots. The clots showed a more tightly woven network of fibres, more fibrin folding, fusing,



**Figure 16:** Brief overview of research chapters and main findings. FBC: full blood count; CRP: C-reactive protein; RBC: red blood cell; PLT: platelet; R: reaction time; K: kinetics; TMRTGG: Time to the maximum rate of thrombus generation; G: shear elastic modulus strength; MA: maximum amplitude; MRTGG: maximum rate of thrombus generation; TGG: total thrombus generation; Px: patient.

and cross-linking. These changes in the fibres of the BCa patient group were confirmed with the findings of the TEG<sup>®</sup>. Specifically related to the increased MA values which indicates increased clot rigidity and strength. Even though there was not any clinical presentation of these changes, it must be noted that this patient group did present with a changed clotting profile that may still have adverse subclinical effects for the patient long-term.

The SEM images revealed notable alterations in the fibres of the BCa group, suggesting a potential formation of micro clots. While these micro clots may not pose the same level of fatality as a thromboembolism, they have the capacity to obstruct microvasculature, thereby contributing to reduced tissue perfusion. Denser networks of fibrin strands are associated with resistance to clot lysis.<sup>328, 329</sup> Common issues that this could lead to would be decreased tissue perfusion, prolonged healing time for wounds, vascular injury, or gradual progression of organ damage.<sup>328, 329</sup>

Taking these findings into consideration, it can be concluded that there is a trend of hypercoagulability associated with this patient group. Furthermore, this presence/potential of hypercoagulability can be augmented when combined with other inflammatory conditions such as high blood pressure, smoking, diabetes, if the cancer becomes metastatic or when patients are put on different treatment plans. This is because these conditions can all have adverse effects on the coagulation system. Diabetic patients often present with a prothrombotic state due to PLT hypersensitivity and hypofibrinolysis.<sup>330</sup> High blood pressure increases coagulation by decreasing fibrinolysis.<sup>331</sup> Since hypertension and inflammation is also proven to be interlinked,<sup>332</sup> the increase in TF expression, decrease in fibrinolytic activity,<sup>333</sup> and PARs-mediated coagulation<sup>98</sup> as seen in inflammatory conditions can also influence and enhance these effects when paired with high blood pressure.

Smoking exacerbates the effects of high blood pressure on coagulation. Sivagangalakshmi et al. showed that smoking alters coagulation profiles by influencing prothrombin time, higher fibrinogen levels, and PLT count. Furthermore, chronic smoking increases PLT aggregability,<sup>334</sup> elevated FXIII and TF pathway inhibitor.<sup>335</sup> Therefore, by combining a condition like this with primary, non-metastatic BCa or if the cancer should become metastatic, it is expected that coagulation will be upregulated. This of course can have detrimental effects that stem from thrombotic events.

Ultimately, this study provided valuable insights into clotting tendencies in this patient group, and it should be emphasised that patients at risk should be monitored and that a treatment targeting fibrin should be considered.

## LIMITATIONS

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With 25 patients in total, this study has a relatively low sample size. This was mainly due to the strict exclusion criteria to limit any confounding factors. A small sample size may have an effect on the statistical power of the obtained results and possibly influence significance. The age range for this study was wide in order to include as many patients as possible. This led to a significant difference between the average ages of the control group compared to the patient group ( $p < 0.0001$ ). Since convenient sampling was used to recruit all the patients, the ages were dependent on the patients admitted during the recruitment period. The difference in age was not excluded as a confounding factor, however age has not been showed to contribute to major changes to blood cells.

## FUTURE STUDIES

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Future studies could include a larger number of patients. For the purpose of identifying the possible contributors to the observed increase in certain TEG<sup>®</sup> parameters, future studies could also focus on measuring the levels of fibrinogen, PLT activation, and/or thrombin generation. In doing so, isolating the procoagulants responsible for increasing the risk of hypercoagulation. In addition, AFM can be used to study RBC rigidity as well as measuring fibrin branching and thickness to investigate the presence of any changes to the formed fibres. This can direct the development of management strategies or targeted anticoagulation therapies. Future avenues of study on this topic might also include only metastatic BCa patients since some studies suggest that metastases might have a greater effect on coagulation as discussed in this dissertation.

The effect of different treatment options may also be worthwhile to explore in order to establish any trends or changes in haematological and clotting properties. While this study only focused on the three most common CNS tumours to limit confounders, future research could also explore the effects of different types of CNS cancers and/lesions. Future research might also include the further subdivision of results based on the age of patients and controls to establish age-related trends as well as patients that have started treatment

plans or patients with comorbidities. C-reactive protein as a biomarker of inflammation can be general, it may therefore be worthwhile exploring other biomarkers that are particularly linked to cancer. Soluble urokinase plasminogen activator (suPAR) is a biomarker that is showing to be promising correlation with inflammation and needs further investigation on its role in oncology.

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## APPENDIX A: ETHICS APPROVAL

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Faculty of Health Sciences

**Institution:** The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

4 July 2022

**Approval Certificate  
New Application**

Dear Miss ZJ Seyfert

**Ethics Reference No.: 232/2022**

**Title: Investigating the hypercoagulability in brain cancer patients by studying the viscoelastic and ultrastructural properties of whole blood**

The **New Application** as supported by documents received between 2022-04-26 and 2022-06-29 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-06-29 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- ∞ Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-07-04.
- ∞ Please remember to use your protocol number (232/2022) on any documents or correspondence with the Research Ethics Committee regarding your research.
- ∞ Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

**Ethics approval is subject to the following:**

- ∞ The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

**On behalf of the FHS REC, Dr R Sommers**

MBChB, MMed (Int), MPharmMed, PhD

**Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria**

*The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)*

Research Ethics Committee  
Room 4-60, Level 4, Tswelopele Building  
University of Pretoria, Private Bag x223  
Gezina 0031, South Africa  
Tel +27 (0)12 358 3084  
Email: despeka.behan@up.ac.za  
www.up.ac.za

Fakulteit Gesondheidswetenskappe  
Lefapha la Disaense eSa Maphelo



Faculty of Health Sciences

**Institution:** The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

20 June 2023

**Approval Certificate  
Annual Renewal**

Dear Miss ZJ Seyfert,

**Ethics Reference No.:** 232/2022 – Line 1

**Title:** Investigating the hypercoagulability in brain cancer patients by studying the viscoelastic and ultrastructural properties of whole blood

The **Annual Renewal** as supported by documents received between 2023-05-29 and 2023-06-14 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-06-14 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- ∞ Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2024-06-20.
- ∞ Please remember to use your protocol number (232/2022) on any documents or correspondence with the Research Ethics Committee regarding your research.
- ∞ Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

**Ethics approval is subject to the following:**

- ∞ The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

**On behalf of the FHS REC, Professor C Kotzé**

MBChB, DMH, MMed(Psych), FCPsych, PhD

**Acting Chairperson:** Faculty of Health Sciences Research Ethics Committee

*The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)*

Research Ethics Committee  
Room 4-60, Level 4, Tswelopele Building  
University of Pretoria, Private Bag x323  
Gezina 0031, South Africa  
Tel +27 (0)12 356 3084  
Email: [deepika.behari@up.ac.za](mailto:deepika.behari@up.ac.za)  
[www.up.ac.za](http://www.up.ac.za)

Fakulteit Gesondheidswetenskappe  
Lefapha la Disaense tsa Maphelo

## APPENDIX B: NHRD APPROVAL

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*Enquiries: Dr JS Mangwane*  
*Tel No: +2712 3452018*  
*Fax No: +2712 354 2151*  
*E-mail: joseph.mangwane@gauteng.gov.za*

**For attention:** janette Bester

**NHRD Ref Number:** GP\_202207\_019

**Re: REQUEST FOR PERMISSION TO CONDUCT RESEARCH AT STEVE BIKO ACADEMIC HOSPITAL**

**TITLE:** Investigating hypercoagulability in brain cancer patients by studying the viscoelastic and ultrastructural properties of whole blood

Permission is hereby granted for the above-mentioned research to be conducted at Steve Biko Academic Hospital. This is done in accordance to the "Promotion of access to information act No 2 of 2000".

Please note that in addition to receiving approval from Hospital Research Committee, the researcher is expected to seek permission from all relevant department. Furthermore, collection of data and consent for participation remain the responsibility of the researcher.

The hospital will not incur extra cost as a result of the research being conducted within the hospital.

You are also required to submit your final report or summary of your findings and recommendations to the office of the CEO.

**STATUS OF APPLICATION: Approvec**

Date: 2022-07-26

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Dr. J S. Mangwane  
Manager: Medical Service

## APPENDIX C: CONTROL INFORMED CONSENT

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**ICD 2 c**

### HEALTHY PARTICIPANT'S INFORMATION & INFORMED CONSENT DOCUMENT

**STUDY TITLE:** *Investigating the viscoelastic and ultrastructural properties of whole blood in brain cancer patients*

**Principal Investigator:** Zenobia Jacomine Seyfert

**Supervisor:** Prof Janette Bester

**Institution:** University of Pretoria

**Daytime telephone number:** 067 388 5225

**DATE AND TIME OF INFORMED CONSENT DISCUSSION:**

dd	month	year

:
Time

## Dear Prospective Research Participant

Dear Mr / Ms / Mrs .....

### 1) INTRODUCTION

You are invited to volunteer for a laboratory-based research study conducted by the Department of Physiology (School of Medicine, Faculty of Health Science). I am doing this research for Master's degree purposes at the University of Pretoria. The information in this document is provided to help you to decide if you would like to participate. Before you agree to take part in this study, you should fully understand what is involved. If you have any questions, which are not fully explained in this document, do not hesitate to ask the researcher. You should not agree to take part unless you are completely happy with the kind of questions that will be asked. It is advised that you inform your personal doctor of your participation in this study.

### 2) THE NATURE AND PURPOSE OF THIS STUDY

The aim of this study is to study the coagulation changes in brain cancer that might indicate thrombotic risk. By doing so we wish to learn more about the clotting behavior of the blood of brain cancer patients which and how that might affect the risk of having blood clots block arteries and/or veins. This will allow us to understand if brain cancer patients in South Africa have changed blood clotting properties. To accomplish this, we will be using specialized microscopes (scanning electron microscope and light microscope) to examine the structure of red blood cells, and platelets. We will also be using equipment that tests the blood clotting properties (thromboelastography and enzyme-linked immunosorbent assay) to determine the degree to which clotting is changed in the blood. These analyses will be done on the blood of brain cancer patients and compared to that of individuals without brain cancer, such as yourself should you decide to participate.

### 3) EXPLANATION OF PROCEDURES AND WHAT WILL BE EXPECTED FROM PARTICIPANTS

This study involves answering some questions regarding your health and any illnesses, examination of yourself, weight and height measurements, and taking some blood samples. One tube of blood will be drawn by a qualified nurse or phlebotomist into a citrate tube, each containing 5 ml of blood (or the equivalent of one teaspoon).

Within four hours of collection, the samples will be analysed and prepared for thromboelastography, light microscopy, and scanning electron microscopy. The blood will then be frozen short term at -80 °C until all the sample recruitment is completed. The samples will also then be used for enzyme-linked immunosorbent assay (cotinine levels).

The blood collection process will only occur once, and no follow-up tests will be required.

### 4) ADDITIONAL USES FOR SAMPLES

The samples that you give to this study principal investigator could one day lead to discoveries using methods and tests not included in this protocol. This may include studying epigenetic changes using quantitative polymerase chain reaction (qPCR) and/or metabolomics using nuclear magnetic resonance (NMR) spectroscopy. This will be used to further study the clotting changes in brain cancer patients compared to healthy individuals in order to identify any abnormalities. These tests may only be identified after the results from this study have been obtained. To that end, we would like to keep the samples for as long as they are deemed useful for research purposes. This research could potentially be used for purposes not specified above for up to five years of collection. However, you may specify a shorter period of time for the study principal investigator to keep the samples.

You have the right to withdraw your consent at any time and may request that the samples you give to the study principal investigator be destroyed. If you choose to do so, contact your study doctor, Zenobia Seyfert, at 067 388 5225. Although you are free to withdraw your consent, it is possible the samples may have already been used for research purposes and data derived from such research will not be destroyed. In that event, the study principal investigator will promptly destroy any remaining samples. Ethical approval will be obtained before any further testing on the residual samples.

We would like you to complete a questionnaire. It will take approximately five minutes. We will collect the questionnaire from you before you leave the ward/ study site. We will be available to help you with the questionnaire. The researcher will keep the completed questionnaires in a safe place to make sure that only people working on the study will have access to it. Please do not write your name on the questionnaire. This will ensure that your answers are kept confidential (so nobody will know what you have answered).

***The questionnaire consists of three parts:***

Part 1: Personal information (age and gender)

Part 2: Medical information (smoking and drinking habits, chronic medications, HIV status)

Part 3: Medical history (chronic medication, contraception, medication/supplements)

**5) RISK AND DISCOMFORT**

The only possible risk and discomfort involved in the blood collection from a vein which could result in bruising, bleeding, and less common infection. For your protection, this procedure will be done under sterile conditions by a medical doctor.

**6) POSSIBLE BENEFITS OF THIS STUDY**

Although you may not benefit directly, the study results may help us to improve the understanding of brain cancer related complications in the future. Many of these tests are routine, and we will be able to treat you, should you have any problems.

**7) ETHICS APPROVAL**

This Protocol was submitted (232/2022) to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, Medical Campus, Tswelopele Building, Level 4-59, Telephone numbers 012 356 3084 / 012 356 3085 and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving humans. A copy of the Declaration may be obtained from the investigator should you wish to review it.

## **8) INFORMATION**

If you have any questions concerning this study, you may contact Miss Zenobia Seyfert. Cell: 0673885225.

## **9) CONFIDENTIALITY**

All records from this study will be regarded as confidential. All participants partaking in this study will be provided an alphanumeric number, e.g. A01. This will ensure the confidentiality of information collected. All results will be published or presented in such a way that it is not possible to identify the participants. The hard copies of all your records will be kept in a locked facility at the Department of Physiology at the University of Pretoria.

## **10) YOUR RIGHTS AS A RESEARCH PARTICIPANT**

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Your participation in this study is entirely voluntary and you may refuse to participate or stop at any time without stating a reason. Your withdrawal will not affect the way you will be treated.

## **11) COMPENSATION**

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You will not be paid to take part in the study. You will also carry no cost for partaking in this study.

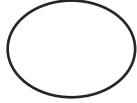
## **12) CONSENT TO PARTICIPATE IN THIS STUDY**

- I confirm that the person requesting my consent to take part in this study has told me about the nature and process, any risks or discomforts, and the benefits of the study.
- I have also received, read and understood the above written information about the study.
- I have had adequate time to ask questions and I have no objections to participate in this study.
- I am aware that the information obtained in the study, including personal details, will be anonymously processed and presented in the reporting of results.
- I understand that I will not be penalised in any way should I wish to discontinue with the study and my withdrawal will not affect my employment or student status.
- I am participating willingly.





## APPENDIX D: CONTROL GROUP QUESTIONNAIRE

Date Captured (dd/mm/yyyy):	Allocated study ID (e.g., A01) (Will be allocated by the investigator)	
<b>PERSONAL INFORMATION</b>		
Sex: <input type="radio"/> male <input type="radio"/> female <input type="radio"/> prefer not to say	Age:	
<b>MEDICAL INFORMATION AND HISTORY</b>		
Do you smoke tobacco or any related product? If yes, for how long?	<input type="radio"/> NO <input type="radio"/> YES _____	
In the past 30 days, have you consumed five/more alcoholic drinks on the same occasion?	<input type="radio"/> NO <input type="radio"/> YES	
Do you have or have you had any of the following conditions?		
<input type="radio"/> Diabetes <input type="radio"/> High blood pressure <input type="radio"/> Heart problems <input type="radio"/> Stroke <input type="radio"/> Arthritis <input type="radio"/> Heart attacks <input type="radio"/> Inflammatory conditions <input type="radio"/> Allergies Please Specify: _____ <input type="radio"/> Other Please specify: _____		
HIV Status:	<input type="radio"/> POSITIVE <input type="radio"/> NEGATIVE <input type="radio"/> UNKNOWN	
<b>MEDICAL HISTORY</b>		
Are you taking any chronic medication? If yes, please specify.	<input type="radio"/> NO <input type="radio"/> YES _____	
Are you taking contraceptives?	<input type="radio"/> NO <input type="radio"/> YES _____	
Have you taken any of the following within the last two weeks?		
<input type="radio"/> Vitamin supplements <input type="radio"/> Corticosteroids <input type="radio"/> Anti-inflammatories <input type="radio"/> Anti-coagulative <input type="radio"/> Other Please specify: _____		

## APPENDIX E: PATIENT INFORMED CONSENT

---

**ICD 2 c**

### **PATIENT'S INFORMATION & INFORMED CONSENT DOCUMENT**

**STUDY TITLE:** *Investigating the viscoelastic and ultrastructural properties of whole blood in brain cancer patients*

**Principal Investigator:** Zenobia Jacomine Seyfert

**Supervisor:** Prof Janette Bester

**Institution:** University of Pretoria

**Daytime telephone number:** 067 388 5225

**DATE AND TIME OF INFORMED CONSENT DISCUSSION:**

dd	month	year

:
Time

**Dear Prospective Research Participant**

Dear Mr / Ms / Mrs .....

**1) INTRODUCTION**

You are invited to volunteer for a laboratory-based research study conducted by the Department of Physiology (School of Medicine, Faculty of Health Science). I am doing this research for Master's degree purposes at the University of Pretoria. The information in this document is provided to help you to decide if you would like to participate. Before you agree to take part in this study, you should fully understand what is involved. If you have any questions, which are not fully explained in this document, do not hesitate to ask the researcher. You should not agree to take part unless you are completely happy with the kind of questions that will be asked. It is advised that you inform your personal doctor of your participation in this study.

**2) THE NATURE AND PURPOSE OF THIS STUDY**

The aim of this study is to study the coagulation changes in neurosurgical conditions (such as brain cancer) that might indicate thrombotic risk. By doing so we wish to learn more about the clotting behavior of the blood of these patients and how that might affect the risk of having blood clots block arteries and/or veins. This will allow us to understand if these patients in South Africa have changed blood clotting properties. To accomplish this, we will be using specialized microscopes (scanning electron microscope and light microscope) to examine the structure of red blood cells, and platelets. We will also be using equipment that tests the blood clotting properties (thromboelastography) to determine the degree to which clotting is changed in the blood. These analyses will be done on the blood of patients with neurosurgical conditions and compared to that of healthy individuals.

**3) EXPLANATION OF PROCEDURES AND WHAT WILL BE EXPECTED FROM PARTICIPANTS**

This study involves answering some questions regarding your health and any illnesses, examination of yourself, and taking some blood samples. One tube of blood will be drawn by a qualified nurse or phlebotomist into a citrate tube, each containing 5 ml of blood (or the equivalent of one teaspoon).

Within four hours of collection, the samples will be analysed and prepared for thromboelastography, light microscopy, and scanning electron microscopy. The blood will then be frozen short term at -80 °C until all the sample recruitment is completed. The samples will also then be used for enzyme-linked immunosorbent assay (cotinine levels).

The blood collection process will only occur once, and no follow-up tests will be required.

**4) ADDITIONAL USES FOR SAMPLES**

The samples that you give to this study principal investigator could one day lead to discoveries using methods and tests included in this protocol. This may include studying epigenetic changes using quantitative polymerase chain reaction

(qPCR) and/or metabolomics using nuclear magnetic resonance (NMR) spectroscopy. This will be used to further study the clotting changes in patients with neurosurgical conditions compared to healthy individuals in order to identify any abnormalities. These tests may only be identified after the results from this study have been obtained. To that end, we would like to keep the samples for as long as they are deemed useful for research purposes. This research could potentially be used for purposes not specified above for up to five years of collection. However, you may specify a shorter period of time for the study principal investigator to keep the samples.

You have the right to withdraw your consent at any time and may request that the samples you give to the study principal investigator be destroyed. If you choose to do so, contact your study doctor, Zenobia Seyfert, at 067 388 5225. Although you are free to withdraw your consent, it is possible the samples may have already been used for research purposes and data derived from such research will not be destroyed. In that event, the study principal investigator will promptly destroy any remaining samples. Ethical approval will be obtained before any further testing on the residual samples.

We would like you to complete a questionnaire. It will take approximately five minutes. We will collect the questionnaire from you before you leave the ward/study site. We will be available to help you with the questionnaire. The researcher will keep the completed questionnaires in a safe place to make sure that only people working on the study will have access to it. Please do not write your name on the questionnaire. This will ensure that your answers are kept confidential (so nobody will know what you have answered).

***The questionnaire consists of three parts:***

Part 1: Personal information (age and gender)

Part 2: Medical information (smoking and drinking habits, chronic medications, HIV status)

Part 3: Medical history (chronic medication, contraception, medication/supplements)

**4) RISK AND DISCOMFORT**

The only possible risk and discomfort involved in the blood collection from a vein which could result in bruising, bleeding, and less common infection. For your protection, this procedure will be done under sterile conditions by a medical doctor.

**5) POSSIBLE BENEFITS OF THIS STUDY**

Although you may not benefit directly, the study results may help us to improve the understanding of neurosurgical conditions (such as brain cancer) related complications in the future. Many of these tests are routine, and we will be able to treat you, should you have any problems.

**6) ETHICS APPROVAL**

This Protocol was submitted (232/2022) to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, Medical Campus, Tswelopele Building, Level 4-59, Telephone numbers 012 356 3084 / 012 356 3085 and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving humans. A copy of the Declaration may be obtained from the investigator should you wish to review it.

## 7) INFORMATION

If you have any questions concerning this study, you may contact Miss Zenobia Seyfert. Cell: 0673885225.

## 8) CONFIDENTIALITY

All records from this study will be regarded as confidential. All participants partaking in this study will be provided an alphanumeric number, e.g. A01. This will ensure the confidentiality of information collected. All results will be published or presented in such a way that it is not possible to identify the participants. The hard copies of all your records will be kept in a locked facility at the Department of Physiology at the University of Pretoria.

## 9) YOUR RIGHTS AS A RESEARCH PARTICIPANT

---

Your participation in this study is entirely voluntary and you may refuse to participate or stop at any time without stating a reason. Your withdrawal will not affect the way you will be treated.

## 10) COMPENSATION

---

You will not be paid to take part in the study. You will also carry no cost for partaking in this study.

## 11) CONSENT TO PARTICIPATE IN THIS STUDY

- I confirm that the person requesting my consent to take part in this study has told me about the nature and process, any risks or discomforts, and the benefits of the study.
- I have also received, read and understood the above written information about the study.
- I have had adequate time to ask questions and I have no objections to participate in this study.
- I am aware that the information obtained in the study, including personal details, will be anonymously processed and presented in the reporting of results.
- I understand that I will not be penalised in any way should I wish to discontinue with the study and my withdrawal will not affect my employment or student status.
- I am participating willingly.
- I have received a signed copy of this informed consent agreement.



\_\_\_\_\_  
Investigator's Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Name of the person who witnessed the  
informed consent (Please print)

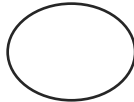
\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of the Witness

\_\_\_\_\_  
Date

Participant Code: \_\_\_\_\_

## APPENDIX F: PATIENT GROUP QUESTIONNAIRE

Date Captured (dd/mm/yyyy):	Allocated study ID (e.g., A01) (Will be allocated by the investigator)	
<b>PERSONAL INFORMATION</b>		
Hospital Number:	Diagnosis:	
Date of Birth (dd/mm/yyyy):	Age:	
Sex: <input type="radio"/> male <input type="radio"/> female <input type="radio"/> prefer not to say		
<b>MEDICAL INFORMATION AND HISTORY</b>		
Do you smoke tobacco or any related product? If yes, for how long?	<input type="radio"/> NO <input type="radio"/> YES _____	
In the past 30 days, have you consumed five/more alcoholic drinks on the same occasion?	<input type="radio"/> NO <input type="radio"/> YES	
Do you have or have you had any of the following conditions?		
<input type="radio"/> Diabetes <input type="radio"/> High blood pressure <input type="radio"/> Heart problems <input type="radio"/> Stroke <input type="radio"/> Arthritis <input type="radio"/> Heart attacks <input type="radio"/> Inflammatory conditions <input type="radio"/> Allergies Please Specify: _____ <input type="radio"/> Other Please specify: _____		
HIV Status:	<input type="radio"/> POSITIVE <input type="radio"/> NEGATIVE <input type="radio"/> UNKNOWN	
<b>MEDICAL HISTORY</b>		
Are you taking any chronic medication? If yes, please specify.	<input type="radio"/> NO <input type="radio"/> YES _____	
Are you taking contraceptives?	<input type="radio"/> NO <input type="radio"/> YES _____	
Have you taken any of the following within the last two weeks?		
<input type="radio"/> Vitamin supplements <input type="radio"/> Corticosteroids <input type="radio"/> Anti-inflammatories <input type="radio"/> Anti-coagulative <input type="radio"/> Other Please specify: _____		

## APPENDIX G: REAGENTS AND EQUIPMENT USED FOR LM, SEM, AND TEG®.

Item	Supplier
<b>Light microscopy</b>	
Zeiss Axio Imager.M2 use	Zeiss Laboratory for Microscopy and Microanalysis, University of Pretoria
Microscope slides	Labocare
Eppendorf single channel Pipette (2-20 µl)	Labcal Solutions cc
Pipette tips (2-20 µl)	Labocare
Methanol	Sigma Aldrich
Mayer's haematoxylin stain	Sigma Aldrich
Eosin stain	Sigma Aldrich
Entellan mounting solution	Sigma Aldrich
Microscope cover glasses (22x40 mm)	Paul Marienfeld GmbH & Co. KG
<b>Scanning electron microscopy</b>	
Zeiss ULTRA Plus FEG-SEM	Zeiss Laboratory for Microscopy and Microanalysis, University of Pretoria
Microscope cover glasses (10 mm)	Lasec
Lyophilized human thrombin	South African National Blood Service
Eppendorf single channel pipette (2-20 µl)	Lasec
Pipette tips (2-20 µl)	Lasec
Phosphate buffered saline (0.01 M)	Sigma Aldrich
Formaldehyde (4%)	Sigma Aldrich
Glass pasteur pipettes	Lasec
Osmium tetroxide (≥99%)	Sigma Aldrich
Ethanol	Sigma Aldrich
Hexamethyldisilane	Sigma Aldrich
Well cell culture plate	Greiner bio-one
Aluminium stubs	Advanced laboratory solutions
Carbon tape	Advanced laboratory solutions
Quorum q150t es carbon coater	Quorum Technologies Ltd. Laboratory for Microscopy and Microanalysis, University of Pretoria
<b>Thromboelastography®</b>	
Haemoscope TEG® analyser	Haemoscope Corporation
Eppendorf single channel pipette (100–1000 µL)	Lasec
Pipette tips (100–1000 µL)	Lasec
TEG® cups	Haemonetics Corporation
Calcium chloride (0.2 M)	Haemonetics Corporation

## APPENDIX H: TURNITIN PLAGIARISM REPORT

### ORIGINALITY REPORT

**10%**

SIMILARITY INDEX

**6%**

INTERNET SOURCES

**7%**

PUBLICATIONS

**4%**

STUDENT PAPERS

### PRIMARY SOURCES

<b>1</b>	<b>Submitted to University of Pretoria</b> Student Paper	<b>3%</b>
<b>2</b>	<b>repository.up.ac.za</b> Internet Source	<b>1%</b>
<b>3</b>	<b>"Posters", Journal of Thrombosis and Haemostasis, 07/2009</b> Publication	<b>&lt;1%</b>
<b>4</b>	<b>atuder.org.tr</b> Internet Source	<b>&lt;1%</b>
<b>5</b>	<b>zombiedoc.com</b> Internet Source	<b>&lt;1%</b>
<b>6</b>	<b>Trauma Induced Coagulopathy, 2016.</b> Publication	<b>&lt;1%</b>
<b>7</b>	<b>"ECR 2019: Book of Abstracts", Insights into Imaging, 2019</b> Publication	<b>&lt;1%</b>
<b>8</b>	<b>dergipark.org.tr</b> Internet Source	<b>&lt;1%</b>
<b>9</b>	<b>www.hindawi.com</b>	