The committee was interested in the topic of your research. The following concerns were mentioned:

1. Do we understand correctly that this is a post-hoc analysis? The committee had concerns regarding the large number of post hoc papers following the ARISTOTLE trial.

RESPONSE: This manuscript is indeed a post-hoc analysis of the ARISTOTLE trial, although the plan was "prospective" in that a detailed proposal was developed prior to any analyses being performed. We respectfully disagree that the large number of "post-hoc" analyses from ARISTOTLE are a problem—in fact, we believe that using a high quality database to address a variety of important questions is a strength of our study and its output. At the same time, we acknowledge that all observational analyses are limited by potential confounding and that when the analyses are driven by selective results of multiple analyses, "multiplicity" is an issue.

Especially in the field of geriatric medicine, polypharmacy has been introduced as a rather "simple" tool for the practicing physician that reflects a patient's extent of comorbidity. In general, polypharmacy has been associated with adverse outcome for many different types of patient populations and, for atrial fibrillation in particular, it has been related to an increased risk of bleeding and mortality (ref 6-17). Whether the effects of a new drug may be consistent with increasing extent of comorbidity is an important clinical issue that we address.

In light of the above we believe that this study addresses an important clinical issue, using some of the best data currently available. We acknowledge that the data from this observational analysis has its limitations and we have addressed this in the limitations section of the manuscript (Page 15).

2. The committee question the use of no/medications as a proxy for comorbidities.

RESPONSE: We appreciate this comment and acknowledge that it is not actually a "proxy," although there is a strong relationship with comorbidities (and their treatments). Although it is likely that differences in prescription patterns may also contribute to a difference in the number of medications used per patient, various reports have documented that polypharmacy/the number of drugs used is a good indicator of the multi-morbidity and thus provides an indication of the frailer patient (ref 5-10). In addition to these reports, our data clearly indicate that with an increasing number of concomitant drugs, the prevalence of nearly all comorbidities increases (Table 1).

Moreover, the simple distinction made upon the number of drugs used has shown to be effective in discriminating high- from low-risk individuals in many different disease settings (ref 5-10). As for atrial fibrillation, polypharmacy has been associated with a higher risk for mortality, and an increased risk for bleeding (ref 9-11, 17), which was confirmed in our study. This is additional evidence to underscore the use of polypharmacy to identify higher-risk, frailer individuals. We acknowledge the limitations related to this 'simplified' stratification and have added this in the Limitations section (Page 15).

3. Another issue is that bleeding risk can be estimated more directly, using HAS BLED, which includes the key risk markers.

RESPONSE: We agree with this comment, but the aim of our study was not to provide a new indicator of bleeding, for which HAS-BLED has been introduced. A previous analysis has demonstrated that the benefit of apixaban over VKA was consistent irrespective of whether the HAS-BLED score was higher or lower than 3 (Lopes et al, Lancet 2012). As published in reference 30, we also demonstrated that the benefit of apixaban was not age dependent (<65 vs. 65-74 vs. ≥75 yrs).

However, among elderly patients there are patients with very little comorbidity, whereas there are also younger patients with significant comorbidity (Discussion Page 13 Lines 314-319).

Similarly, there are patients with HAS-BLED scores <3 with important comorbidity, and patients with HAS-BLED scores ≥3 with only moderate comorbidity. In fact, HAS-BLED does not cover the variety of comorbidity that the number of concomitant medications does. The latter reflects the number of organ systems affected, whereas HAS-BLED is a mere indicator of bleeding risk.

Above mentioned distinctions are important clinical issues for which we raise awareness with the present manuscript. Polypharmacy could serve as a "simple tool" to facilitate this differentiation.

With our categorization based on the number of drugs used, we identify a group of patients with a higher extent of comorbidity and adverse outcome; this corroborates with various previous reports on polypharmacy (ref 5-10).

Polypharmacy identifies the frail patient, which means that—irrespective of potential drug-drug interactions—regulatory mechanisms in these patients are often suboptimal and may alone increase the risk of complications. For example, fluid and electrolyte balance regulation in these patients is impaired and may more easily result in marked renal function deterioration in case of, for example, diarrhea, which may then cause side effects. Thus, polypharmacy is not intended as a new marker of bleeding, but it does appear to represent a general marker of frailty and adverse outcome (both bleeding, stroke, and mortality) which may be related to a differential drug response to anticoagulation therapy.

4. You make statements such as "Regarding intracranial bleeding, the benefit of apixaban remained consistent across tertiles" in the results section. We would prefer it you presented the data and left the interpretation for the discussion (the abstract also has multiple similar statements).

RESPONSE: We agree that interpretation of the data should be placed in the Discussion. Therefore, we have changed the text in the Results and Abstract, as suggested.

5. Why were some patients taking so many medications? Was this because of significant comorbidities? Or does this may reflect prescription patterns?

   Patients in the US, for example, were taking more concomitant medications. Was this because they were sicker? Or because of lack of coordination among specialists? Is number of medications a marker for comorbidity?

RESPONSE: These are important points. As pointed out, it is striking that patients are taking so many medications. There are various reasons for the use of so many medications. First of all, there was a clear association between the number of medications and the number of comorbidities per patient, indicating that patients with more medications have more medical problems and tend to be "sicker" (Table 1 and Supplementary Table 1). This association was consistent, both for US and non-US patients.

In addition, we also observed specific prescription patterns, in that patients in the US were taking substantially more medications when compared with non-US patients (Supplementary Table 2 A,B). This in and of itself is a striking finding and raises the question as to whether patients are being "overmedicated" in the US. Moreover, this is why we adjusted for the country of randomization.

To address your questions in more detail, patients in the US had a greater number of comorbidities per patient (see Table below). In addition, they received almost twice as many drugs, regardless of the degree of comorbidity. We touched on this in the Discussion section of the original submission (P11, first paragraph). We now have revised this section to highlight this important topic (Page 8-9 Lines 182-187; Page 12 Lines 286-289).

We can provide this table as a Supplementary Table if the committee wishes; we chose not to do so in our original submission to keep the focus on our main message, which is irrespective of the country of randomization.

Table. Association between number of organs/systems affected by comorbidities and by medications

A. Patients from the United States

		Number of concomitant medications			
		0 - 5	6 - 8	9 or more	**Total**
Number of organs/systems affected by comorbidities	0 – 1 (n, %)	110 (32.8%)	126 (37.6%)	99 (29.6%)	335 (10.5%)
	2 – 3 (n, %)	242 (16.3%)	515 (34.8%)	724 (48.9%)	1481 (46.2%)
	4 or more (n, %)	80 (5.8%)	264 (19.0%)	1045 (75.2%)	1389 (43.3%)
	Total (n, %)	432 (13.5%)	905 (28.2%)	1868 (58.3%)	3205 (100%)

B. Patients from non-U.S. countries

		Number of concomitant medications			
		0 - 5	6 - 8	9 or more	**Total**
Number of organs/systems affected by comorbidities	0 – 1 (n, %)	1848 (61.4%)	989 (32.8%)	174 (5.8%)	3011 (23.7%)
	2 – 3 (n, %)	2935 (41.4%)	2845 (40.1%)	1309 (18.5%)	7089 (55.8%)
	4 or more (n, %)	612 (23.5%)	957 (36.8%)	1033 (39.7%)	2602 (20.5%)
	Total (n, %)	5395 (42.5%)	4791 (37.7%)	2516 (19.8%)	12702 (100%)

6. What medications were involved? Were there differences in medications between regions and patients? Were specific drug combinations deleterious?

RESPONSE: We categorized all drugs according to the World Health Organization (WHO) system (Table 2). Separate data for US and non-US patients are presented in Supplementary Tables (Supplemental Tables 2A and 2B). In our original discussion, we noted the issue of prescription differences, supported by the aforementioned tables, and we have now tried to emphasize this more throughout the revised manuscript.

It is beyond the scope of this manuscript to tease out the association of specific drug combinations with outcomes in detail (Table 2, Supplemental Tables 2A and 2B, Results section page 11 Lines 245-255, Discussion page 14 Lines 335-339).

It is also beyond our scope to describe and study in detail the impact of regional differences. We agree that this is an important dimension of the issue and therefore we have adjusted not only for age and sex, but also for country of randomization. In our revised manuscript, we followed this methodological approach.

We agree that potential differences in drug classes/drug combinations between treatment groups may affect the observed outcomes as well (see reviewer 1, # 1). In the Results section we have added that there were no differences across tertiles between the two treatment groups (apixaban and warfarin) with regard to the rate of the different drug classes (Page 8 Lines 172-174). As part of a more in depth approach, we found that the observed interaction on major bleeding remained consistent after sequential exclusion of one of the respective drug classes.

Moreover, we acknowledge that additional information for certain types of drugs (antiplatelet agents, NSAIDs, prednisone) may add clarity for the interpretation of our current study question (Table 2). Finally, data on interacting drugs and NOACs are limited, and potential drug-drug interactions may well be of importance in patients with a higher number of concomitant medications. In this context, we also studied whether drugs interacting with apixaban and/or warfarin could have contributed to the observed interaction on major bleeding (Table 2 and 4, Results section page 11 Lines 245-255, Discussion page 14 Lines 335-339).

7. Please explain the clinical value for general readers in more detail.

RESPONSE: In our revised manuscript we have further described the key clinical messages from our analysis.

First, we report that polypharmacy is common in this population and that it is a marker of the extent of comorbidity. Notably, we demonstrate that polypharmacy is associated with adverse outcomes (stroke/SE, major bleeding, mortality) in patients with atrial fibrillation treated with anticoagulants. Either the multiple concomitant drugs themselves or the related frailty (with higher risks of stroke and bleeding and death) could result in a differential response to anticoagulation drugs.

Second, we found that the efficacy of apixaban compared with warfarin was consistent with increasing numbers of medications.

The fact that we found an interaction between the safety of apixaban (versus warfarin) and polypharmacy suggests that the benefit of apixaban may be more substantial in patients on fewer medications who have less comorbidity.

In the Introduction and Discussion, we have further highlighted the clinical messages of our report:
"In an era with a growing elderly population, the difference between biological and chronological age is an important clinical issue. Polypharmacy may aid to get a first impression of how 'old'/'young' your patient truly is. In addition, this report may contribute to the appreciation that polypharmacy may be related to a different response to anticoagulant drugs, which has also been suggested by Piccini et al (Circulation 2015) concerning rivaroxaban."

In the Results and Discussion, we also add new, clinically relevant information to the scant data available on drug-drug interactions regarding the CYP3A4 and P-gp pathways. We also provide data on combinations with selected medications that may increase bleeding risk (antiplatelet agents, NSAIDs, prednisone).

8. For p values above the conventional 0.05 cut-off there is little justification for quoting more than one decimal place.

RESPONSE: We agree and we have changed the manuscript accordingly.

9. The committee thought the statistical analysis was appropriate and the conclusions justified.

RESPONSE: We would like to thank the committee for this remark.

** Comments from the external peer reviewers**

Reviewer: 1

Comments: Drs Focks et al report on a sub-analysis of the Artistole trial with special reference in reference to the polypharmacy used in older patient groups. This is a relevant topic with increasing importance with ageing of population. To get an objective information access to the original patient charts/documentation is required.

Major comments:

1. Dividing of medication in quartiles is one possibility to assess the topic of the paper. However, interaction of medication between themselves and – more importantly – with Apixaban or Warfarin is not respected. This is a major drawback of the study and the results of the presented analysis are week if not misleading.

RESPONSE: As requested, we now report results according to drugs known to interact with apixaban. Drugs known to inhibit the P-glycoprotein (P-gp) as well as the cytochrome P450 3A4 (CYP3A4) pathways, which would increase the apixaban plasma concentration, were used by 20.9% of the patients using apixaban, with increasing rates across tertiles. However, no significant association between use of these drugs and major bleeding was observed (Table 4).

As for drugs known to potentiate warfarin, these were used by 21.1% of the patients assigned to warfarin. Interestingly, specific use of these drugs was (likewise) not independently associated with major bleeding during clinical follow-up.

In addition, we reported the drug-drug interactions with antiplatelet agents, NSAIDs, and predisone in more detail in the revised manuscript (Table 2 and Page 11).

While a detailed description of the broad variety of potential drug-drug interactions is beyond the scope of this manuscript, we agree that the manuscript is strengthened with more information on the key interaction drugs that we now include.

In the context of your question, we also refer to a previous report on polypharmacy and the impact of non-vitamin K antagonists (ref 10).

2. The decrease of the benefit of Apixaban over Warfarin by increase of medications. The contrary would have been expected because of the larger number of interaction of drugs with Warfarin compared to Apixaban. The conclusion does not take in account this results by just repeating the result and without giving an interpretation or hypothesis (see second to last sentence in Abstract).

RESPONSE: This is an interesting observation and we agree that our findings are counterintuitive. However, polypharmacy not only indicates a greater number of drugs, as we noted before, it also reflects the extent of comorbidity (1), i.e. 'frail patients,' and identifies a high-risk group for adverse outcomes. In addition to drug-drug interactions (2), this could also contribute to a differential response to anticoagulation drugs.

As for potential interpretations and explanations, we provided additional information and discussion in the revised manuscript (Pages 13-14). We discuss that extracranial major bleeding

seems the key driver of the observed interaction, as can be seen in Figure 2. While the hazard ratio for reduction in intracranial bleeding (with apixaban vs. warfarin) became lower with more medications, the hazard ratio for GI bleeding increased across tertiles. We performed additional analyses to investigate whether the observed interaction on major bleeding was consistent, irrespective of the use of ASA/NSAIDs/prednisone. Finally, we added information on potentiating drug combinations for warfarin and apixaban. Especially in the case of the latter, this information is novel, and of importance for clinical practice (Discussion Page 14 Lines 335-339).

Finally, we redrafted the abstract to incorporate more interpretation/hypothesis, as you requested. We mentioned the two potential reasons for a differential response to anticoagulation therapy (1. extensive comorbidity, and 2. drug-drug interactions) and we provided results (1. COPD/GI-history/renal impairment, and 2. CYP3A4-GPG inhibitors) that relate to these issues.

3. Tables 1 and 2 pool the data of the groups A and W. This is incorrect and all texts and tables and interpretations should be eliminated.

RESPONSE: We agree that the study drug allocation was not stratified according to the number of drugs used, but in a large trial there is unlikely to be substantial imbalance in large baseline subgroups. Hence, there is a risk of baseline differences between treatment groups which could have influenced our findings.

Our primary aim with Tables 1 and 2 was to demonstrate:1) that comorbidity in the total study population increased with the number of drugs used; and 2) how the use of each of the various classes of drugs increased across tertiles.

In this context, we feel that elimination of the tables will not facilitate the interpretation of certain key messages of our manuscript. The two points mentioned above are irrespective of the anticoagulant used (apixaban or warfarin). Notwithstanding, we should provide more details on potential differences in baseline characteristics between the treatment groups apixaban and warfarin across tertiles.

Therefore, we have provided a new table containing information on the baseline characteristics including the extra columns for apixaban and warfarin to more fully disclose the data (Supplementary Table 1). We believe that this table contains information that is not essential for the manuscript and is overly complex if added to main Table 1; thus we propose to add it as supplementary table, although we defer to the editors as to whether it should be displayed as the main Table 1.

After careful consideration within our study group we did not change the layout of Table 2 to ensure a reader-friendly presentation, and easier interpretation of the intended message. We opted for additional textual information in the Results section to inform the reader that there were no substantial differences in baseline characteristics between apixaban and warfarin across the three groups. We hope that this satisfies the main concerns (Page 8, Line 172-174).

4. Table 3 and related text passages: the statement that with increasing number of medications the effect of A over S reduces on bleeding event does not hold for all bleedings analysed: is holds for “major being” with p 0.0173 and for “major or clinically relevant non-major bleeding” with p 0.0475. For the other events this statement does not hold. Moreover, it is unclear, if “major bleeding” were counted twice for the two groups of bleeding. It would be clearer, if the second “major or clinically relevant non-major bleeding” would refer only to “clinically relevant non-major bleeding” (which is also in accordance with the literature).

RESPONSE: It is true that the treatment interaction holds true only for "major bleeding" and for "major or non-major clinically relevant bleeding." We have reviewed the manuscript and removed any statements that could have been interpreted otherwise.

As for the second remark, major bleeding was not counted twice, but was incorporated both in "major bleeding" as well as in the outcome variable "major or non-major clinically relevant bleeding." We have followed your suggestion and reported "non-major clinically relevant bleeding" as a separate outcome variable (See Table 3, Figure 2).

5. What is the outcome on mortality?

RESPONSE: As far as all-cause death is concerned, we observed an increasing risk across the tertiles (Table 3) with no signs of a treatment interaction (Figures 1 and 2). This was also briefly depicted in the Results section of the original submission. However, this was placed under the subheading "Stroke and Systemic Embolism," which we now have changed to "Efficacy Outcomes" (Page 9).

Minor comments:

1. Figure 1 for supplementary

RESPONSE: In the revised manuscript we have moved Figure 1 of the original submission to the Supplementary Materials.

2. Many non-significant results. Shorten or add to supplementary

RESPONSE: We agree that the tables contained many different outcomes with non-significant results. We tried to make a more concise presentation of the key data. Moreover, to improve the readability, we have converted Table 4 of the original submission into Figures 1 and 2.

3. Table 1 A and 1 B: same as for Tables 1 and 2. Information misleading because treatment groups are pooled

RESPONSE: As discussed above, we respectfully disagree that pooling the treatment arms is a misleading way to present the baseline data, given the large subgroups with no substantial differences in any of these characteristics and with objectives being largely non-anticoagulant specific.

For Table 1 depicting the baseline characteristics, we now provide separate columns for apixaban and warfarin use in Supplementary Table 1. For the sake of reader-friendly presentation, we did not change the layout of the other tables, and address the issue of baseline differences between treatment groups in the text of the Results section. As such, we did not change Supplementary Tables 2A and 2B, but provided additional textual information. As stated above, Supplementary Table 1 shows that, for each of the three tertiles, there are no relevant baseline differences between the treatment groups.

Reviewer: 2

Comments:
This is another post-hoc analysis of the ARISTOTLE trial, a phase III trial that investigated apixaban vs. warfarin for stroke prevention in atrial fibrillation (AF). Here, the authors aimed at analyzing the effect of polypharmacy on risk of stroke and bleeding. In detail, the authors analyzed the correlation of the "crude" number of concomitant medications with clinical outcomes.
The results of the analysis are not unexpected, as both ischemic and bleeding complications occurred more often in patients treated with a higher number of concomitant medications. The efficacy results

seem to be similar to result of the original publication of the ARISTOTLE trial, while bleeding complication were similar for apixaban and warfarin with increasing number of co-medications.

Here are my specific comments:

1. Was this analysis predefined in the study protocol of ARISTOTLE?

RESPONSE: The current analysis was not predefined in the original ARISTOTLE study protocol which we acknowledge in the Limitations section of the revised manuscript (Page 15). However, the analysis plan for this was developed prior to the analysis being performed, such that it was not a "data-driven" analysis.

2. The number of co-medications seem to be a surrogate for co-morbidities/frailty, and risk scores for stroke and/or bleeding. Explanation of the added value of this particular analysis would be important.

RESPONSE: We acknowledge the limitations of using the number of medications as a surrogate for comorbidities (Page 15). However, despite the fact that differences in the threshold to prescribe drugs between doctors may affect the classification in individual cases, several reports have demonstrated on a group level that polypharmacy is associated with more comorbidity and a higher risk of adverse outcome, also in atrial fibrillation (ref 5-17). In addition, our data clearly indicate that with an increasing number of concomitant drugs, the prevalence of nearly all comorbidities increases (Table 1). In this context we are confident that the number of drugs can be used as an indicator of the extent of comorbidity.

The aim of our study was not to provide a new predictor or model of bleeding, for which HAS-BLED has been introduced. A previous analysis has demonstrated that the benefit of apixaban over VKA was irrespective whether the HAS-BLED score was higher or lower than 3 (Lopes et al, Lancet 2012). As stated in reference 30, we also demonstrated that the benefit of apixaban was not age dependent (<65 vs. 65-74 vs ≥75 yrs).

However, among elderly patients, there are patients with hardly any comorbidity, whereas there are also younger patients with significant comorbidity (Page 13). In analogy, there are patients with HAS-BLED scores <3 with important comorbidity, and patients with HAS-BLED scores ≥3 with only moderate comorbidity.

We are of the opinion that awareness for this clinical nuance is an important issue for daily clinical practice in an era with a growing elderly population. In addition, this report may contribute to the appreciation that polypharmacy may be related to a different response to anticoagulant drugs.

3. The definition of polypharmacy and classification based on the crude number of concomitant medications is somehow arbitrary:

RESPONSE: This is an important remark. A uniform definition of polypharmacy does not exist. However, our approach was not arbitrary, it was based on a common approach of dividing our data into tertiles to allow exploration of polypharmacy across categories that are sufficiently large to avoid the hazards of small subgroups. Most reports define polypharmacy as the use of more than 5 drugs (ref 21; Page 15 Lines 369-373). However, in patients with atrial fibrillation, about 60% of patients would classify for polypharmacy with this definition, in our population it would be 76.5%. In view of this, we prospectively decided to categorize our patient population into tertiles. This approach, rather than dichotomizing the population based upon a certain cut-off for polypharmacy, also gives us more detailed information about: 1) potential differences in the sort and number of baseline comorbidities with an increasing number of drugs; and 2) potential differences in the relative risk reductions of apixaban vs. warfarin with regard to efficacy and safety with increasing numbers of drugs.

4. It would be interesting to have detailed information of the medications (class of drugs, reason of prescription, etc.).

RESPONSE: As for the class of drugs, we categorized all drugs according to the World Health Organization (WHO) drug classification system which is depicted in Table 2 (we refer to the WHO website for more detailed information [ref. 23], to get an impression of the kind of drugs listed per class). Unfortunately, we do not have information on the appropriateness of medication use, which we reported in the Limitation section (Page 15). There were no relevant differences between apixaban and warfarin users across tertiles, with regard to the different classes of drugs used. Although it was beyond the scope of this manuscript to perform a detailed study on the different medications used, we acknowledge that our manuscript can be strengthened with some additional information. We decided to provide more detailed information on often used drugs such as aspirin/NSAIDs/prednisone and to study the impact of potentiating drugs of warfarin and apixaban (Table 2 and 4, Results page 11, Discussion page 14).

5. Also analysis according co-medications that inhibit p-glycoprotein and/or the CYP-system would be clinically useful - instead categorizing patients according to the "crude" number of co-medications.

RESPONSE: As requested, we now report information on drugs known to interact with apixaban and with warfarin. Drugs known to concomitantly inhibit the P-glycoprotein (PGP) as well as the cytochrome P450 3A4 (CYP3A4) pathway, which would increase the apixaban plasma concentration, were used by 20.9% of the patients using apixaban. However, no association between these drugs and major bleeding was observed. As for drugs known to potentiate warfarin, this was used by 21.1% of the patients assigned to warfarin. Interestingly, also these drugs were not associated with major bleeding during clinical follow-up.

We have described this in the Results section of the manuscript (Table 4 and Page 11) and added information to Table 2 on interacting medication.

6. One limitation is that only the baseline number of co-medications was analyzed, changes during follow-up might be relevant. However, this is a clinical trial setting and I do not expect a relevant effect of changes during follow-up on the effects of anticoagulation on stroke prevention and bleeding.

RESPONSE: We agree that the changes in concomitant medications would be interesting, but patients with polypharmacy driven by chronic medical conditions do not often have a dramatic reduction in the number of drugs (Limitations, Page 15). We also share your thought that this information would probably not influence the effects of anticoagulation on stroke prevention and bleeding.

Follow-up information was only available for the oral anticoagulants. We reported the permanent study drug discontinuation rates and observed an increase across tertiles of number of medications but without difference between treatment groups (Table 3, Results Page 11).