The association of Hypertension and Diabetes Pharmacotherapy with COVID-19 severity and immune signatures : An observational study

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Background

Coronavirus disease 2019 (COVID-19) in patients with co-existing diabetes and hypertension is associated with an increased risk of severe infections and mortality (1). There is a paucity of data on the association of pharmacotherapy of these conditions with COVID-19 disease severity.

Methods:

We conducted a retrospective, observational cohort study of 717 patients with PCR-confirmed COVID-19 who were hospitalised at the National Centre of Infectious diseases (NCID), Singapore up to April 15, 2020. Data collected included demographics, co-morbidities, concomitant medications, and clinical outcomes. Primary outcomes were hypoxia (requirement for supplemental oxygen to maintain blood oxygen saturations >93%), intensive care unit (ICU) admission, mechanical ventilation or death. The study was conducted in accordance with institutional guidelines; study protocols were reviewed and approved, by the Ministry of Health, Singapore and the institutional ethics committee (ref: 2012/00917).

Plasma immune mediator concentrations were measured using multiplex microbead-based immunoassay: Cytokine/Chemokine/Growth Factor 45-plex Human ProcartaPlex^{TM} panel 1 (ThermoFisher Scientific).

We used the modified Poisson regression approach (2) to calculate the relative risk (RR) for the association between requirement for supplementary oxygen, ICU admission, mechanical ventilation, and death with diabetes, hypertension and pharmaco-therapeutics. RR was adjusted for demographics, comorbidities and co-medications. Multivariable linear regression models were used to assess the association between inflammatory markers, and the use of medications. All statistical tests were two-sided, and statistical significance was taken as $p<0.05$. All statistical analyses were performed using Stata version 15.

Cytokine levels across groups were compared using two-tailed Mann Whitney *U* test. . Statistical analyses and data plotting were performed using GraphPad Prism (Version 8.2.1)

Results :

In this cohort, 139 (19.4%) had hypertension and 76 (10.6%) had type 2 diabetes mellitus (Table 1). Hypoxia was reported in 91 (12.7%), ICU admission in 47 patients (6.6%), mechanical ventilation in 25 (3.5%) and 12 patients (1.67%) died.

Diabetes and hypertension were associated with hypoxia (Diabetes: adjusted RR [aRR] 1.76, 95% confidence interval [CI] 1.18-2.63; Hypertension: aRR 1.96, 95% CI 1.25-3.07) and ICU admission (Diabetes: aRR 2.17, 95% CI 1.23-3.83; Hypertension: aRR 2.23, 95% CI 1.17-4.24).

In the hypertension subgroup ACE-I treatment was associated with a lower risk of ICU admission (aRR 0.26, 95% CI 0.10-0.68) and mechanical ventilation (aRR 0.09, 95% CI 0.02-0.36), whereas ARBs were associated with a higher risk of ICU admission (aRR 2.19, 95% CI 1.08- 4.43) (Table 1).

In the diabetes subgroup, patients receiving a DPP4i were at higher risk of ICU admission (aRR 4.07, 95% CI 1.42-11.66) (Table 1). SGLT2i was associated with a marginally lower risk of mechanical ventilation (aRR 0.03, 95% CI 0.00-0.70). Sensitivity analysis adjusting for BMI, HbA1c, systolic and diastolic blood pressure at baseline showed similar results (Table 1). The results also remained materially unchanged when the analysis was confined to patients on metformin. Since 88% of patient with diabetes received metformin it was not possible to analyse its effects.

In the hypertension subgroup, the use of ARB was associated with higher C-reactive protein concentrations (β-19.0; p=0.038), white blood cell counts (β-1.14, p=0.006) and neutrophil counts (β-1.24, p=0.002) when adjusted for age, gender and ethnicity.

Diabetes patients on DPP4i had significantly lower brain derived neurotrophic factor (BDNF). Hypertension patients on ARB treatment had significantly higher MCP-1 and IP-10 concentrations compared to non-ARB users. (Figure 1).

Longitudinal profiling of these cytokines showed that IFN-γ, IL-18, IL-15 and BDNF trends were mixed during disease progression in diabetes patients with no differences with treatment

whilst MCP-1 and IP-10 concentrations continued to remain significantly higher in hypertension ARB users vs non-users.

Discussion:

We found that patients with COVID-19 and diabetes or hypertension had more severe infections with a higher requirement for oxygen and ICU admission. These results are similar to other larger observational studies reported globally (1).

As severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses the angiotensin converting enzyme -2 (ACE-2) for entry into alveolar cells (3), there has been concern about the effect of RAS modulators on COVID-19 severity. The concerns about possible increased viral transmission due to upregulation of the ACE-2 enzyme expression has been recently debunked as a large population-based study showed no correlation with viral transmission(4,5).

In the hypertension cohort, ACE-I was associated with better outcomes. In a recent meta-analysis, which included 16 studies involving 24,676 patients, they similarly found that the use of ACE-I associated with better disease outcomes (6). Conflicting results have been reported from various centers as highlighted in another meta-analysis(7). Many studies have combined ACE-I and ARBs into one group as they have patients on a combination and thus, they are unable to study the effects individually. Our cohort did not have a single patient who were taking both ACE-I and ARB allowing us to individually assess these medications.

Since ACE-I blocks the formation of angiotensin-II completely, it can lead to a counter up-regulation of the ACE-2 pathway which can protect against respiratory failure (8). On the other hand, ARB blocks the AT1R with a concomitant increase in angiotensin-II. Angiotensin-II can act through other receptors like (AT3R and AT4R) which are not completely blocked by ARBs (9). Serum angiotensin-II concentrations have been seen to correlate with viral load and lung injury in COVID-19 (10). Moreover, angiotensin-II can induce endothelial cell synthesis of MCP-1 and IP-10 , pro-inflammatory cytokines which has been identified to be strongly associated with severe COVID-19 (10,11). This is consistent with our observations that the patients receiving ARB treatment have higher levels of plasma MCP-1 and IP-10 compared to patients receiving ACE-I treatment, or neither.

In the diabetes cohort, we found that patients on DPP4-inhibitors were more likely to require ICU admission. In COVID-19, the adaptive T-cell immune response is thought to play a significant role in determining disease severity. If the T-cell and B-cell response are not effective, immune cells accumulate in the lungs, causing overproduction of pro-inflammatory

cytokines and damaging inflammation (12). DPP4 inhibitors have been associated with the suppression of T cell proliferation response which may result in a less effective immune response to the virus (13). In support of this hypothesis, we did observe a significant decrease in brain derived neurotrophic factor (BDNF), which is important for T-cell maturation (Figure 1). In BDNF knockout mice and in conditional knockout mice lacking BDNF (specifically in this lymphoid subset), diminished T-cell cellularity in peripheral lymphoid organs has been demonstrated suggestive of a critical paracrine and autocrine role in thymocyte development (14).

This study is limited by its single center design, observational study design and relatively small sample size.

We found a beneficial signal for ACE-I and a deleterious signal for ARB and DPP4-inihibitors with evidence of deleterious immune signatures. Data from larger observational cohorts and ongoing randomized controlled trials is urgently needed to confirm or refute these findings.

Author contributions: RD conceptualized the study, performed data analysis, data interpretation, literature review and wrote the manuscript. WT and BEY conceptualized the study, performed data collection, and critically reviewed the manuscript. SWF, WCT, YHC, LR and LN performed the microbead immunoassay, analyzed, interpreted and reported the results for the T cell response. LWA performed data analysis and interpretation. DEKC : data interpretation and critical review of manuscript. DCL conceptualized the study, data interpretation and critical review of manuscript. All authors reviewed the final manuscript.

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Conflict of Interest: All authors state that they have no conflict of interest with regards to this manuscript.

Data Availability : The datasets are available from the corresponding author on reasonable request.

References

1. Cen Y, Chen X, Shen Y, Zhang X-H, Lei Y, Jiang W-R, Xu H-T, Chen Y, Zhu J, Zhang L-L, Liu Y-H. Risk factors for disease progression in mild to moderate COVID-19 patients- a multi-center observational study [published online ahead of print, 2020 Jun 8]. *Clin Microbiol Infect.* 2020;S1198-743X(20)30341-4.

2. Zou G. A Modified Poisson Regression Approach to Prospective Studies with Binary Data. *Am J Epidemiol.* 2004; 159:702–6.

3. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.* 2020;46:586-590

4. Sama IE, Ravera A, Santema BT, van Goor H, ter Maaten JM, Cleland JGF, Rienstra M, Friedrich AW, Samani NJ, Ng LL, Dickstein K, Lang CC, Filippatos G, Anker SD, Ponikowski P, Metra M, van Veldhuisen DJ, Voors AA.. Circulating plasma concentrations of ACE2 in men and women with heart failure and effects of renin–angiotensin–aldosterone inhibitors. *Eur Heart J*. 2020; doi:10.1093/eurheartj/ehaa373

5. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin-Angiotensin-Aldosterone System Blockers and the Risk of Covid-19. *N Engl J Med.* 2020;382:2431-2440.

6. Pirola CJ, Sookoian S. Estimation of Renin-Angiotensin-Aldosterone-System (RAAS)-Inhibitor effect on COVID-19 outcome: A Meta-analysis. *J Infect*. 2020 ; S0163-4453 :30329-7.

7. Grover A, Oberoi M. A systematic review and meta-analysis to evaluate the clinical outcomes in COVID-19 patients on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers [published online ahead of print, 2020 Jun 15]. *Eur Heart J Cardiovasc Pharmacother.* 2020; pvaa064. doi:10.1093/ehjcvp/pvaa064

8. Tikellis C, Thomas MC. Angiotensin-converting enzyme (ACE2) is a key modulator of the renin angiotensin system in health and disease. *Int J Pept.* 2012 ; 256294. doi :10.1155/2012/256294

9. Numaguchi Y, Ishii M, Kubota R, Morita Y, Yamamoto K, Matsushita T, Okumura K, Murohara T. Ablation of angiotensin IV receptor attenuates hypofibrinolysis via PAI-1 downregulation and reduces occlusive arterial thrombosis. *Arterioscler Thromb Vasc Biol.* 2009; 29:2102- 2108.

10. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Jing Y, Wang Z, Li J, Li J, Feng C, Zhang Z, Wang L, Peng L, Chen L, Qin Y, Zhao D,Tan S, Yin L, Xu J, Zhou C, Jiang C, Liu L. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci*. 2020 ;63 :364-374.

11. Ide N, Hirase T, Nishimoto-Hazuku A, Ikeda Y, Node K. Angiotensin II increases expression of IP-10 and the renin-angiotensin system in endothelial cells. *Hypertens Res.* 2008 ; 31 :1257-67.

12.Vardhana SA, Wolchok JD. The many faces of the anti-COVID immune response. *J Exp Med.* 2020;217:e20200678.

13.Kitagawa N, Hamaguchi M, Majima S, Fukuda T, Kimura T, Hashimoto Y, Tanaka M, Yamazaki M, Nakamura N, Fukui M. Dipeptidyl peptidase- 4 inhibitors have adverse effects for the proliferation of human T cells. *J Clin Biochem Nutr*. 2018; 63: 106–12.

14. Linker RA, Lee DH, Flach AC, Litke T, Brandt JVD, Reichardt HM, Lingner T, Bommhardt U, Sendtner M, Gold R, Flügel A, Lühder F . Thymocyte-derived BDNF influences T-cell maturation at the DN3/DN4 transition stage. *Eur J Immunol*. 2015;45:1326-1338.

Table 1. Association of the use of anti-hypertensives and oral hypoglycaemic agents with clinical indicators of COVID-19 severity in patients with hypertension (n=139) and diabetes (n=76).

Hypertension : ^Adjusted for age, gender, ethnicity, use of other anti-hypertensive medications, diabetes medications and statins. Since no patients on ACE-I was on ARB and vice versa these were evaluated in 2 separate models including only ACE-I or ARB.
Diabetes: ^Adjusted for age, gender, ethnicity, use of other anti-hypertensive medications, other diabetes medications (except metformin) and statins. [#]Additionally adjusted for HbA1c, systolic and diastolic blood pressure and body mass index. RR, relative risk; aRR, adjusted relative risk; CI, confidence interval. * $p<0.05$

Figure 1. Immune signatures of COVID-19 patients with Diabetes Mellitus and Hypertension treated with DPP-4i, ACE-I or ARB. Plasma fractions were isolated from the blood of COVID-19 patients without diabetes mellitus (DM) and hypertension (HTN) (n = 12), with DM (n = 17; 7 patients on DPP-4i treatment), or with HTN (n = 19; 2 patients on ACE-I treatment and 7 patients on ARB treatment). Concentrations of immune mediators were quantified using a 45-plex microbead-based immunoassay. Cytokine levels were measured on the first plasma samples collected upon hospitalization (Median 8 days post illness-onset (PIO)). Immune mediator profiles that are differently regulated in patients receiving anti-diabetic (DPP-4i) of anti-hypertensive (ACE-I or ARB) treatment are illustrated as scatter plots. Statistical analyses were performed using Mann-Whitney U test (*$P < 0.05$). Cytokine levels of patient samples that are not detectable are presented the value of logarithmic transformation of Limit of Quantification (LOQ). Cytokine level for healthy controls (HC) (n = 23) is indicated by the blue dotted line.

Figure 1

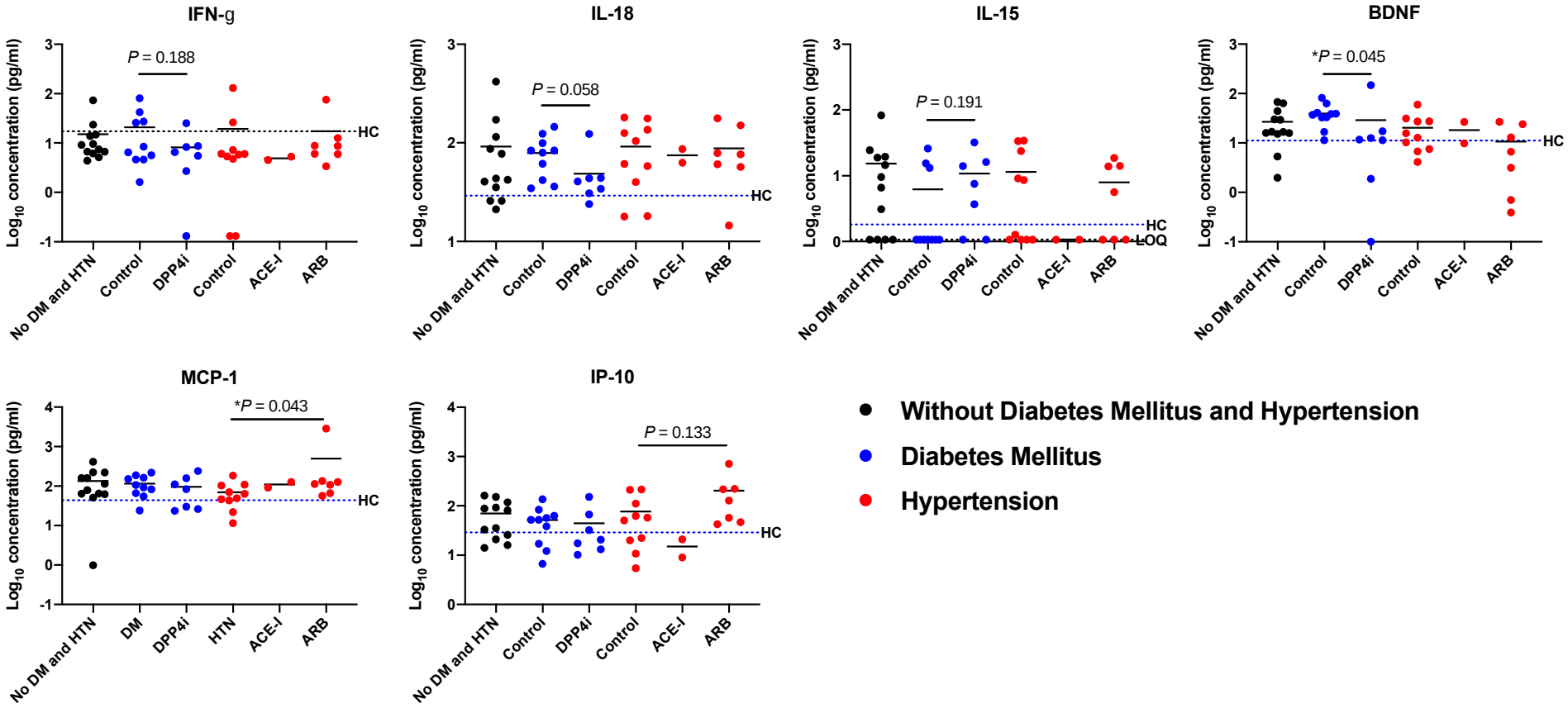

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Table 1

Table 1: Association of the use of anti-hypertensives and oral hypoglycaemic agents with clinical indicators of COVID-19 severity in patients with hypertension (n=139) and diabetes (n=76)

	Univariable analysis		Multivariable analysis^		Multivariable analysis 2[#]	
	RR (95% CI)	**P value**	**aRR (95% CI)**	**P value**	**aRR[#]**	**P value**
Hypertension Subgroup (n=139)						
Angiotensin converting enzyme inhibitor (ACE-I) (n=28)						
Supplementary oxygen	0.85 (0.47,1.53)	0.588	0.72 (0.36,1.46)	0.367		
ICU admission	0.48 (0.15,1.47)	0.197	0.26 (0.10, 0.68)	0.006*		
Mechanical ventilation	0.28 (0.04, 2.08)	0.215	0.09 (0.02, 0.36)	0.001*		
Death	1.32 (0.28,6.23)	0.725	0.50 (0.08,3.24)	0.468		
Angiotensin Receptor Blocker (ARB) (n=62)						
Supplementary oxygen	1.40 (0.90,2.17)	0.135	1.40 (0.88, 2.21)	0.153		
ICU admission	2.24 (1.11,4.50)	0.024*	2.19 (1.08, 4.43)	0.029*		
Mechanical ventilation	1.86 (0.70, 4.97)	0.214	1.88 (0.56, 6.35)	0.309		
Death	1.24 (0.32,4.79)	0.753	2.87 (0.41,20.0)	0.287		
Calcium Channel Blocker (CCB) (n=68)						
Supplementary oxygen	1.38 (0.88, 2.15)	0.159	1.60 (0.99, 2.58)	0.056		
ICU admission	0.90 (0.46,17.6)	0.769	0.96 (0.44, 2.11)	0.924		
Mechanical ventilation	0.91 (0.35,2.39)	0.854	1.08 (0.37, 3.19)	0.884		
Death	1.04 (0.27,4.03)	0.950	1.94 (0.33,11.43)	0.465		
Beta-blocker (BB) (n=35)						
Supplementary oxygen	1.12 (0.69,1.82)	0.634	1.21 (0.78, 1.89)	0.394		
ICU admission	0.99 (0.46,2.13)	0.981	1.35 (0.62, 2.93)	0.443		
Mechanical ventilation	1.08 (0.37,3.19)	0.888	1.42 (0.47, 4.28)	0.537		
Death	0.99 (0.21,4.71)	0.990	0.94 (0.24, 3.63)	0.927		
Diuretics (n=18)						
Supplementary oxygen	0.90 (0.45,1.80)	0.758	0.73(0.35, 1.52)	0.402		
ICU admission	1.46 (0.63,3.36)	0.373	0.76 (0.29, 2.02)	0.586		
Mechanical ventilation	1.68 (0.52,5.4)	0.384	0.99 (0.23,4.32)	0.991		
Death	6.72 (1.83,22.64)	0.004*	1.20 (0.11,13.04)	0.882		
Diabetes Subgroup (n=76)						
DPP4-inhibitors (n=27)						
Supplementary oxygen	1.81 (1.05, 3.13)	0.032*	2.00 (0.98, 4.09)	0.056	1.80 (0.89, 3.61)	0.100
ICU admission	3.33 (1.38, 8.04).	0.008*	4.07 (1.42, 11.66)	0.009*	5.14 (1.49, 17.70)	0.009*
Mechanical ventilation	1.81 (0.49,6.74)	0.373	2.54 (0.43, 14.99)	0.304	60.2 (3.17, 1140.1)	0.006*
SGLT2-inhibitors (n=16)						
Supplementary oxygen	1.36 (0.75,2.48)	0.308	0.92 (0.40, 2.10)	0.835	0.85 (0.34, 2.09)	0.717
ICU admission	1.56 (0.64,3.81)	0.327	1.07 (0.30, 3.76)	0.922	1.19 (0.26,5.52)	0.825
Mechanical ventilation	0.54 (0.07,4.10)	0.548	0.03 (0.00, 0.70)	0.029*	0.00 (0.00, 0.25)	0.015*
Sulfonylureas (n=33)						
Supplementary oxygen	1.00 (0.57,1.75)	0.990	0.95 (0.48,1.90)	0.885	0.99 (0.48,2.05)	0.980
ICU admission	1.16 (0.50,2.69)	0.733	1.35 (0.50,3.68)	0.557	1.05 (0.37,3.00)	0.922

Mechanical ventilation	1.30 (0.35,4.87)	0.694	3.55 (0.46, 27.33)	0.223	8.68 (1.0,75.2)	0.050

Hypertension : ^Adjusted for age, gender, ethnicity, use of other anti-hypertensive medications, diabetes medications and statins. Since no patients on ACE-I was on ARB and vice versa these were evaluated in 2 separate models including only ACE-I or ARB.

Diabetes : ^Adjusted for age, gender, ethnicity, use of other anti-hypertensive medications, other diabetes medications (except metformin) and statins. [#]Additionally adjusted for HbA1c, systolic and diastolic blood pressure and body mass index. RR, relative risk; aRR, adjusted relative risk; CI, confidence interval. * $p<0.05$

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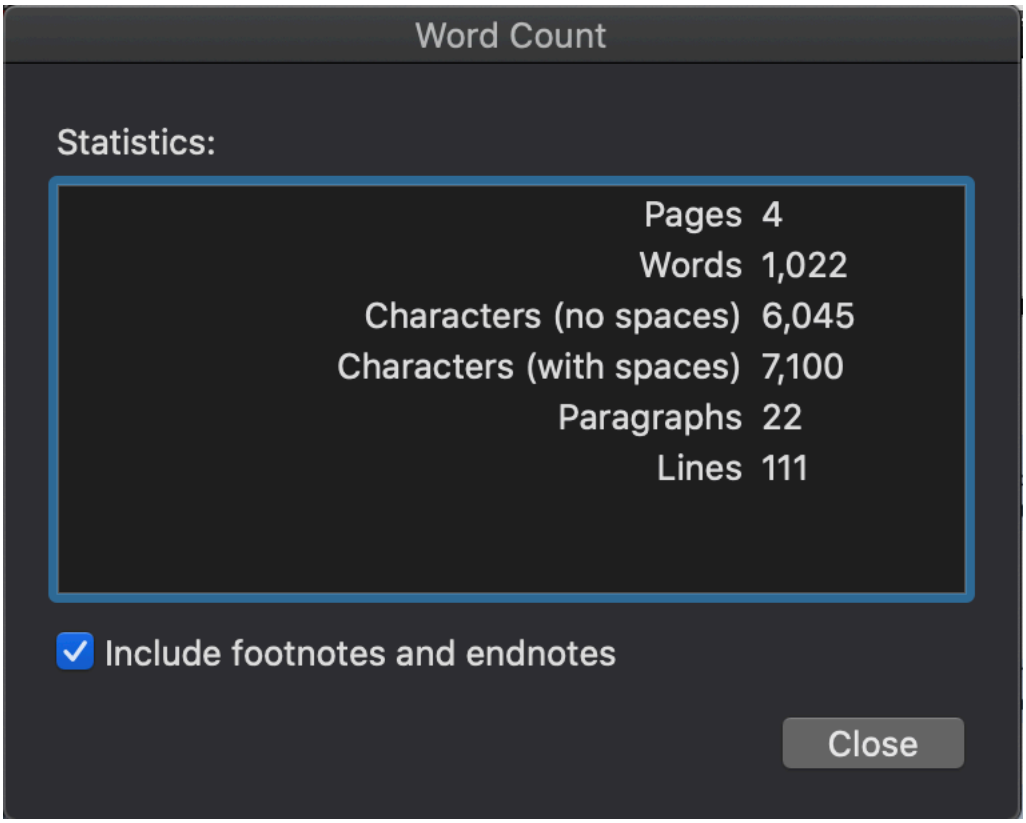
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