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Autism spectrum disorders in adults and the autonomic nervous system: Heart rate variability markers in the diagnostic procedure

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ABSTRACT

The diagnostic assessment of autism spectrum disorders (ASD) in adults is a challenging and time-consuming procedure. In order to address the lack of specialised health-care professionals and improve the waiting time, we aimed to identify specific electrocardiogram (ECG) derived Heart Rate Variability (HRV) parameters that could be used for diagnostic purposes. 152 patients were diagnosed based on a standardised clinical procedure and assigned to one of three groups: ASD ($n = 56$), any other psychiatric disorder (OD) ($n = 72$), and patients with no diagnosis (ND) ($n = 24$). Groups were compared using ANOVA. Discriminative power of biological parameters and the clinical assessment were compared using receiver operating characteristic curves (ROCs). Patients with ASD showed reduced parasympathetic and increased sympathetic activity compared to ND. The accuracy determined by the area under the curve (AUC) of the biological parameters for discrimination between ASD vs. pooled OD/ND was 0.736 (95% CI = 0.652-0.820), compared to .856 (95% CI = 0.795-0.917) for the extensive clinical assessment. Our results confirmed the dysregulation of the autonomic nervous system in ASD with reduced parasympathetic and increased sympathetic activity as compared to ND. The discriminative power of biological markers including HRV was considerable and could supplement less sophisticated clinical assessments.

1. Introduction

Autism spectrum disorders (ASD) are defined as developmental disorders with impairments concerning communication, social interaction, and reduced flexibility, often reflected in restricted and repetitive behaviours and reduced adaptability to environmental changes (World Health Organization, 2016). However, the appearance and severity of the deficits varies significantly between individuals, thus posing a major challenge to current diagnostic procedures. Several factors such as gender, age, intelligence, and the individual history of life experience contribute to the heterogeneous appearance of these disorders, especially in adults (Haker et al., 2016). Moreover, patients with ASD often suffer from psychiatric comorbidities (Buck et al., 2014; Ghaziuddin and Zafar, 2008; Mazzone et al., 2012; Vannucchi et al., 2014). Overlapping symptoms with other disorders and the lack of information on infantile development and behaviour can complicate the diagnostic assessment even further (Mannion and Leader, 2013).

Although there are several tools to assess the core deficits of ASD clinically, most of them are known to show less sensitivity identifying adults with higher functioning and milder forms of autism, and less specificity when discriminating from other psychiatric disorders in clinical settings (Baghdadli et al., 2017; Wigham et al., 2019). One of them is the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 1999), which is considered the "gold-standard" instrument in the diagnostics of ASD. While it has been proven to be a valid and reliable instrument in the diagnostics of ASD in children and adolescents (De Bildt et al., 2004; Gray et al., 2008; Lord et al., 2000; Noterdaeme et al., 2000; Papanikolaou et al., 2009), there are mixed findings concerning the use of the ADOS Module 4 for adults. The main shortcomings of this tool are found in the differentiation between ASD and other psychiatric disorders, especially in complex clinical settings (Bastiaansen et al., 2011; Conner et al., 2019; Maddox et al., 2017; Wigham et al., 2019), and in its higher sensitivity for the "male-type" of autism, causing females with ASD and/or milder symptoms to remain undetected

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(Adamou et al., 2018).

Ultimately, the final diagnostic classification still relies on the interpretation and evaluation of experienced clinicians. In clinical practice, trained and experienced clinicians for ASD in adults are scarce, which often leads to an extended waiting time and poses a second challenge to diagnostics (Brugha et al., 2011; Taylor and Marrable, 2011). The waiting time for the years 2019 and 2020 in the specialised unit of the Psychiatric University Hospital of Zurich amounted in average to 24 weeks. Reports from the United Kingdom suggest similar delays (National Health Service, 2021). Therefore, potentially affected individuals can often not be examined and diagnosed in a timely manner due to the resource-demanding diagnostic procedure (Rutherford et al., 2016). This in turn often leads to a delayed onset of supportive measures and increases the level of suffering for the individual (Renty and Roeyers, 2006). Hence, less time-consuming and more reliable methods to supplement the clinical diagnostic procedures would be desirable.

In the last decades, biomarkers such as biological parameters reflecting the activity of the autonomic nervous system (ANS) have arisen as a promising way of improving psychiatric diagnoses and underpinning them with physiological evidence (Singh and Rose, 2009). Heart rate variability (HRV) as a measure of ANS activity is known as a promising biomarker in psychiatry and can easily be assessed by recording an electrocardiogram (ECG). It is therefore widely accessible, cost-effective, and practicable in most clinical settings. HRV is defined as the varying interval between two consecutive heartbeats over a certain period of time (Rompelman et al., 1977) and has initially been studied in the context of cardiovascular diseases and autonomic balance (Akselrod et al., 1981; Kleiger et al., 1987; Pomeranz et al., 1985; Saul et al., 1990; Schwartz and De Ferrari, 2011; Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). It is now known as a well-established electrophysiological correlate of the functional status of the ANS (Shaffer and Venner, 2013). Further research subsequently showed an association between HRV and emotional regulation (Appelhans and Luecken, 2006, 2008; Geisler et al., 2010; Thayer and Lane, 2000; Visted et al., 2017) and several psychiatric conditions (Alvares et al., 2016; Birkhofer et al., 2005; Yang et al., 2010). This indicates that HRV might reflect the connection between the heart and the brain (McCraty and Shaffer, 2015; Olbrich et al., 2011; Thayer et al., 2012; Thayer and Lane, 2009).

Even though the underlying pathomechanism remains unclear, ASD has consistently been associated with dysregulations of the ANS (Cheng et al., 2020). If we consider the lack of flexibility and adaptability as a core symptom of ASD and the HRV as an index for flexibility and adaptability to stressors, HRV understandably arises as a promising biomarker for the diagnostics of ASD. Accordingly, studies conducted on paediatric ASD populations revealed a reduced HRV compared to typically developing peers under resting-state condition (Ellenbroek and Sengul, 2017; Ming et al., 2005; Neuhaus et al., 2014). While there is a growing body of literature suggesting parasympathetic underactivity in ASD (Benevides and Lane, 2015; Bujnakova et al., 2016), findings concerning sympathetic activity in ASD are in sum inconsistent (Bujnakova et al., 2016, 2017; Kushki et al., 2013; Legiša et al., 2013; Levine et al., 2012; O'Haire et al., 2015; Schaaf et al., 2015).

For adult populations, reduced HRV in adults with ASD compared to healthy neurotypical cohorts have been reported (Thapa et al., 2019). Moreover, a meta-analysis based on 34 studies investigating HRV in individuals with ASD stated that first, the baseline HRV and HRV reactivity under social stress are significantly lower in individuals with ASD compared to subjects without ASD. Second, respiratory sinus arrhythmia (RSA), a commonly used index for parasympathetic activity, seems to be lower in individuals with ASD compared to subjects without ASD during baseline. Third, only high frequency activity (HF) at baseline and other baseline parasympathetic indices revealed a significant intergroup difference when compared with controls. Differences for total variability, were not significant (Cheng et al., 2020).

However, individuals attending a specialised clinic for an ASD assessment usually do so driven by psychological strain due to mental health symptoms and are thus not comparable to healthy neurotypical control groups often reported in studies. Accordingly, when it comes to diagnostics in clinical settings, patients with ASD have to be differentiated from patients with other psychiatric disorders, and even from the "borderline" group of individuals who subjectively experience similar symptoms but at the end of an extensive assessment still do not meet the criteria for any psychiatric disorder. Furthermore, the eventual goal of investigating possible biomarkers is to aid clinical practice in real-world settings (Abi-Dargham and Horga, 2016).

Thus, in order to improve these difficulties in clinical settings, this study aimed to fill in the research gap by investigating HRV in a valuable "real-life" sample of patients referred to a diagnostic ASD assessment by health-care professionals. By doing so, we aimed to identify HRV parameters that could be used for diagnostic preselection by health-care professionals not specialised in ASD, or to supplement less sophisticated clinical assessments.

Based on the aforementioned literature, we first predicted that individuals with ASD would show reduced HRV compared to both patients with other psychiatric diagnoses and patients not meeting the criteria for any psychiatric disorder. Second, we specifically expected patients with ASD to show reduced parasympathetic activity as an expression of reduced HRV in comparison to patients with other diagnoses or patients with no diagnosis. For this purpose, we analysed HRV parameters reflecting the parasympathetic and sympathetic branches of the ANS by comparing individuals with ASD with individuals suffering from other psychiatric disorders and patients not meeting the criteria for any psychiatric disorder.

2. Materials and methods

2.1. Design and sample

We retrospectively analysed the HRV parameters of 153 patients who underwent the ASD diagnostic assessment at the specialised outpatient clinic for ASD in adults at the Psychiatric University Hospital of Zurich between 2018 and 2020. Every patient attending the clinic for a diagnostic assessment who provided written informed consent, was included in the study. The diagnostic assessment consisted of at least three sessions with explorative clinical interviews and third-party assessments administered by specialised clinicians. Whenever possible, parents or primary carers were interviewed to assess the infantile development. The questionnaire used was based on ADI-R (Rutter et al., 2003), but shortened due to time-restrictions arising from the clinical framework. Additionally, self-reported questionnaires were completed at home. During a fourth session at the clinic, a 15 min resting-state electroencephalogram (EEG) and ECG was recorded in order to exclude organic causes. In order to account for stress-induced effects, this procedure was highly standardised, providing equal conditions and allowing for comparability between groups.

Based on this complex and standardised clinical assessment, the sample was divided into three groups, the first one being the group eventually diagnosed with an ASD (ASD) according to ICD-10 criteria (F84.0 Autistic Disorder, F84.1 Atypical Autism or F84.5 Asperger's Syndrome) ($n = 56$); the second one being the group diagnosed with any other psychiatric disorder (OD) according to ICD-10 criteria ($n = 72$); and the third group consisting of patients eventually not meeting the criteria for any psychiatric disorder (ND) as per ICD-10 ($n = 24$). Due to its "borderline-nature" the ND group was of particular interest in terms of differential diagnoses. Patients in this group reached out for a diagnostic assessment due to a subjective experience of mental health symptoms, or at least a subjective feeling of otherness. However, they could not be categorised as either healthy controls, nor psychiatric population, which poses new specific difficulties in the differentiation from the ASD group.

A detailed description of the diagnoses of the OD and comorbidities

of the ASD group can be found in Table 1. To provide a realistic representation of the clinical challenges, the only exclusion criterion applied was persistent cardiac arrythmia, as evaluated by a physician. Demographics and clinical characteristics were retrieved from the patients' clinical records.

2.2. ECG recording and processing

The ECG was simultaneously recorded with a resting-state EEG, lasted 15 min, and was part of the usual clinical procedure for the ASD diagnostic assessment. It was designed as a pure resting condition and did not involve any tasks or movements. Patients were seated in a slightly leaning position in a sound, and light-attenuated room set at a temperature of 22 °C. The ECG was recorded with Polaris.one (ver. 2.1.0.0) software solutions and an amplifier (model JE-921A) by Nihon Kohden Europe GmbH (2012). After cleaning the attachment areas on the skin, reusable silver chloride (Ag/AgCl) cup electrodes filled with conductive gel (OneStep AbrasivePlus) were placed. A lead I configuration with the negative electrode attached to the right forearm and the positive electrode affixed to the left arm was used. Sampling rate was set to 200 Hz and band-pass filtered between (0.16 and 15 Hz).

BrainVision Analyzer (ver. 2.2.0) was used to identify the 15 min resting-state section and extract the ECG signal from the complete EEG/ECG recording. The edf-files were then uploaded to KUBIOS HRV (ver. 3.4) (Tarvainen et al., 2020) to allow for visual inspection and artifact correction.

The ECG recordings had already been evaluated by a physician during the diagnostic procedure in terms of pathological anomalies before being included in the study. Thus, one ECG of an initial sample of 153 patients had to be excluded from the analysis due to a persistent cardiac arrhythmia. An automatic filter based on an algorithm which detects abnormal and ectopic heart beats was applied. The detection method used for this algorithm is based on time-varying thresholds estimated from distribution of successive RR-interval differences combined with a novel beat classification scheme (Lipponen and Tarvainen, 2019). Remaining R-peak artifacts were then corrected manually.

2.3. Measures

2.3.1. HRV parameters

HRV data was analysed in accordance with the Guidelines for Reporting Articles on Psychiatry and HRV (Quintana et al., 2016). The following HRV parameters were calculated for the time domain: mean heart rate in beats per minute (mean HR bpm), standard deviation of R–R intervals (SDNN) in ms., and the square root of the mean squared differences between successive R–R intervals (RMSSD) in ms. In the frequency domain, Fast Fourier transform was used to calculate the absolute power in ms^2, and the relative power in percent of high frequency (HF 0.15–0.4 Hz), low frequency (LF 0.04.-0.15 Hz), very low frequency (VLF 0.0–0.04 Hz), as well as their respective logarithmic values in order to achieve approximative normality of distribution. Additionally, LF power in normalised units (n.u.) and total power in ms^2 was calculated. Further parameters included the stress index, the parasympathetic nervous system (PNS) index, and the sympathetic nervous system (SNS) index (Tarvainen et al., 2014), which have been found to be sensitive measures for the ANS, and in particular for cardiovascular stress, parasympathetic, and sympathetic activity (Olbrich et al., 2021).

Table 1
Detailed description of comorbidities found in the ASD group and diagnoses of the OD group.

	ASD ($n = 56$)			OD ($n = 72$)	
	n	%		n	%
Comorbidities **(ICD-10)**			**Maindiagnosis (ICD-10)**		
None	38	67.9	F32.1, F33.0, F33.1, F33.4	19	26.6
F33.0, F33.1, F33.2	9	16.1	F60.0, F61	16	22.4
F42.0, F42.2	4	7.2	F42.0, F42.2	12	16.8
F50.0, F50.9	2	3.6	F90.0	7	9.8
F61	2	3.6	F40.1, F41.1, F41.2	5	7
F64.0	1	1.8	F43.2	4	5.6
			F21	3	4.2
			F06.7, F07.0	2	2.8
			F45.2, F45.41	2	2.8
			F84.8	1	1.4
			F12.1	1	1.4

Note. ASD = Autism spectrum disorders. *OD* = Other Diagnoses. *ICD-10* = International Classification of Diseases – 10.

2.3.2. Standard clinical procedure with self-reported measures and third-party assessments

The usual diagnostic assessment includes self-reported questionnaires such as the Autism Quotient (AQ) (Baron-Cohen et al., 2001), the Empathizing Quotient (EQ) (Baron-Cohen and Wheelwright, 2004), the Systemizing Quotient (SQ) (Baron-Cohen et al., 2003), the Toronto Alexithymia-Scale (TAS-20) (Bagby et al., 1994), the International Personality Disorder Examination (IPDE) (Loranger, 1997), and the Beck Depression Inventory II (BDI-II) (Beck et al., 1961). While the AQ assesses abnormal or autistic-like behaviour, the EQ measures the every-day understanding of minds, and the SQ evaluates the drive to analyse or construct systems. The TAS-20 measures three facets of alexithymia: difficulties identifying and describing one's own emotions, and externally oriented thinking. All the scales mentioned are part of the usual set of questionnaires administered for the diagnostic ASD assessment and therefore also address differential diagnoses. Hence, the IPDE is used to assess and diagnose personality disorders and the BDI-II provides a measure for the severity of depression.

Third-party assessments include the Faux-Pas Recognition Task (Stone et al., 1998), the Social Attribution Task (SAT) (Klin, 2000), and the Reading the Mind in the Eyes Test (RMET) (Baron-Cohen et al., 2001). The SAT provides scores for social cognition competences, the Faux-Pas Task assesses the Theory of Mind (ToM), and the RMET provides a measure for "mentalising" skills and social sensitivity. The values from the RMET ranged from 0 to 36, whereas the results of the Faux-Pas Test ranged from 0 to 53. Faux-Pas Test values were subsequently categorised into three groups consisting of "underperformance" (0–39), "average" (40–48), and "good performance" (49–53). Furthermore, we provided an equivalent categorization of the qualitative performance. This qualitative assessment was based on the clinical impression and feedback of the individual about the strategies used for mastering the test. For example, patients with a high quantitative score in the Faux-Pas Test, due to little to no mistakes in their answers, but reporting a very systematic approach, high effort in analysing the situations, or answering mainly based on personal experiences with comparable mistakes had a "below-average" qualitative Faux-Pas Score. The results of the SAT were categorised in terms of qualitative performance only ("underperformance", "average", "good performance"). For the self-reported questionnaires, the numerical results of each test and their subscales were used for the analysis as interval scaled variables.

2.4. Statistical analysis

Demographic and clinical characteristics, self-reported measures and third-party assessments were compared using ANOVAs with the corresponding *F*-tests and Pearson's chi-squared test (χ^2). For the comparison of the HRV parameters between the three groups one-, and two-way ANOVAs were used. To ensure normalised data distribution HRV variables were log-transformed. Post-hoc comparisons were Bonferroni corrected. Pearson correlations were performed to analyse the relationship between age, self-reported measures, third-party assessments and HRV parameters. Due to its explorative nature no corrections for multiple correlations were applied. Finally, receiver operating

characteristic curves (ROC) were calculated to compare the standard diagnostic assessment with biological parameters including HRV in terms of diagnostic sensitivity and specificity. As test variables we used either single variables or a combination of variables based on binary logistic regression analyses.

3. Results

3.1. Characteristics of participants

We examined 152 patients, of which 101 were male and grouped them by diagnosis (ASD, other psychiatric diagnoses, no psychiatric diagnosis) (Table 2). Bonferroni-corrected post hoc comparisons revealed that patients diagnosed with ASD (34.07, $SD = 10.01$, $p = .001$) and patients diagnosed with other psychiatric diseases (35.33, $SD = 12.55$, $p = .002$) were significantly younger than patients with no psychiatric diagnoses (44.41, $SD = 9.24$). As shown by the frequencies cross-tabulated in Table 2, the relation between diagnosis and sex was significant, $\chi^2(2, n = 152) = 16.327$, $p < .001$. A chi-square test of independence also revealed a significant association between diagnosis and the referring site, $\chi^2(10, n = 152) = 22.748$, $p = .012$.

3.2. Between-group comparisons of self-reported measures and third-party assessments

As expected, Bonferroni-corrected post hoc comparisons revealed that patients diagnosed with ASD (37.69, $SD = 5.48$) had significantly higher AQ values than patients with other diagnoses (33.11, $SD = 7.46$, $p = .001$) and patients with no diagnosis (31.33, $SD = 8.11$, $p = .001$). Unsurprisingly, the mean AQ values of patients with other diagnoses and patients with no diagnosis did not differ significantly ($p = .832$). No significant differences between groups were found for EQ, SQ, Alexithymia, BDI-II, RMET or Faux-Pas Test sumscore.

A significant relationship was found between diagnosis and qualitative Faux-Pas Test rating, $\chi^2(4, n = 149) = 30.030$, $p < .001$, and diagnosis and qualitative SAT rating, $\chi^2(4, n = 151) = 38.341$, $p < .001$, but not for diagnosis and quantitative Faux-Pas Test rating.

3.3. Between-group comparisons of HRV parameters

A significant difference in the mean SNS value between the diagnostic groups ($F(2, 148) = 3.074$, $p = .049$) was found (Table 3). Bonferroni-corrected post hoc comparisons revealed that patients with ASD (0.77, $SD = 1.47$, $p = .04$) showed significantly higher SNS values than patients with no diagnosis (−0.07, $SD = 0.85$, $p = .044$). However, patients with ASD and patients with other diagnoses (0.57, $SD = 1.45$) did not significantly differ from each other in terms of SNS values ($p = 1.000$), nor did patients with no diagnosis and patients with other diagnoses ($p = .162$). There was also a statistically significant difference between groups in the mean HR values ($F(2, 148) = 4.301$, $p = .015$). Bonferroni-corrected post hoc comparison showed that patients with ASD had a significantly higher heart rate (71.50, $SD = 10.79$), than patients with no diagnosis (63.79, $SD = 8.07$, $p = .012$). However, patients with ASD and patients with other diagnoses (69.43, $SD = 11.53$) had similar mean HR values ($p = .856$) as did patients with no diagnosis and patients with other diagnoses ($p = .085$). Two-way ANOVAs were conducted to calculate the effect of diagnosis and sex on the HRV parameters and the effect of diagnosis and age on the HRV parameters. No significant interaction effects were found.

3.4. Correlations

Explorative Pearson correlations between age, self-reported measures, third-party assessments and HRV parameters were calculated.

Table 2
Between-group comparisons of demographic and clinical characteristics.

	ASD (n = 56)	*Other diagnoses (n = 72)*	*No diagnosis (n = 24)*				
Age	*M (SD)*				*F*	η_p^2	*p*
	34.07_a *(10.01)*	35.33_a *(12.55)*	44.41_b *(9.24)*		7.690	.094	.001**
Sex	*n (%)*			*Total (%)*	*df*	χ^2	*p*
male	26 *(46.4)*	55 *(76.4)*	20 *(83.3)*	101 *(66.4)*	2	16.327	<.001***
Education	*n (%)*			*Total (%)*	*df*	χ^2	*p*
Compulsory education	11 *(19.6)*	19 *(26.4)*	2 *(8.3)*	32 *(21.1)*	4	8.873	.064
Apprenticeship	25 *(44.6)*	39 *(54.2)*	11 *(45.8)*	75 *(49.3)*			
Graduate degree	20 *(35.7)*	14 *(19.4)*	11 *(45.8)*	45 *(29.6)*			
Use of stimulants or beta blockers	*n (%)*			*Total (%)*	*df*	χ^2	*p*
None	49 *(87.5)*	65 *(90.3)*	24 *(100)*	138 *(90.8)*	4	5.997	.199
Stimulants	7 *(12.5)*	5 *(6.9)*	0 *(0)*	12 *(7.9)*			
Beta blockers	0 *(0)*	2 *(2.8)*	0 *(0)*	2 *(1.3)*			
Family history	*n (%)*			*Total (%)*	*df*	χ^2	*p*
No psychiatric diagnoses	18 *(32.1)*	17 *(23.6)*	10 *(41.7)*	45 *(29.6)*	8	14.025	.081
Suspected ASD diagnosis	6 *(10.7)*	3 *(4.2)*	4 *(16.7)*	13 *(8.6)*			
Confirmed ASD diagnosis	2 *(3.6)*	11 *(15.3)*	3 *(12.5)*	16 *(10.5)*			
Other psychiatric diagnoses	28 *(50)*	40 *(55.6)*	7 *(29.2)*	75 *(49.3)*			
n/a (adopted)	2 *(3.66)*	1 *(1.4)*	0 *(0)*	3 *(2)*			
Referring site	*n (%)*			*Total (%)*	*df*	χ^2	*p*
Psychologist	10 *(17.9)*	10 *(13.9)*	1 *(4.8)*	21 *(13.8)*	10	22.748	.012*
Psychiatrist	14 *(25)*	20 *(27.8)*	2 *(8.3)*	36 *(23.7)*			
During in-patient stay	0 *(0)*	3 *(4.2)*	0 *(0)*	3 *(2)*			
Physician	4 *(7.1)*	0 *(0)*	1 *(4.2)*	5 *(3.3)*			
Personal initiative, family or partner	24 *(42.9)*	38 *(52.8)*	20 *(83.3)*	82 *(53.9)*			
Other	4 *(7.1)*	1 *(1.4)*	0 *(0)*	5 *(3.3)*			

Note. ASD = Autism spectrum disorders, *n* = number, *M* = mean, *SD* = Standard Deviation, *F* =F-Test, η_p^2 = partial eta squared, *df* = degrees of freedom, χ^2 = Pearson's chi-squared test, *$p < .05$, **$p < .01$, ***$p < .001$. Means with differing subscripts within rows are significantly different at the $p < .01$ based on Bonferroni-corrected post hoc comparisons.

Table 3
Between-group comparisons of HRV parameters.

	ASD ($n = 56$)	Other diagnoses ($n = 71$)	No diagnosis ($n = 24$)				
	M (SD)			*df*	*F*	η_p^2	*p*
Artifacts %	.92 *(1.62)*	.90 *(1.63)*	1.05 *(1.07)*	2	.079	.001	.924
PNS	−.40 *(1.09)*	−.28 *(1.11)*	.06 *(.91)*	2	1.599	.021	.206
SNS	$.77_a$ *(1.47)*	$.57_{a,b}$ *(1.45)*	$-.07_b$ *(.85)*	2	3.074	.040	.049*
Stress index	12.06 *(5.58)*	11.59 *(5.57)*	9.86 *(2.99)*	2	1.500	.020	.227
SDNN	37.43 *(17.67)*	38.58 *(18.42)*	39.87 *(15.15)*	2	.171	.002	.843
Mean HR	71.5_a *(10.79)*	$69.43_{a,b}$ *(11.53)*	63.79_b *(8.07)*	2	4.301	.055	.015*
RMSSD	37.06 *(22.88)*	37.01 *(21.26)*	38.01 *(16.23)*	2	.021	.000	.979
VLF ms^2	59.65 *(59.99)*	70.62 *(72.56)*	86.46 *(65.14)*	2	1.382	.018	.254
LF ms^2	805.65 *(788.66)*	879.15 *(967.36)*	1030.54 *(1133.28)*	2	.487	.007	.615
HF ms^2	742.65 *(1021.88)*	713.91 *(804.45)*	593.54 *(462.22)*	2	.263	.004	.769
VLF log.	3.71 *(0.89)*	3.88 *(.86)*	4.19 *(.78)*	2	2.615	.034	.077
LF log.	6.22 *(1.03)*	6.28 *(1.07)*	6.56 *(.84)*	2	.952	.013	.388
HF log.	5.97 *(1.25)*	5.89 *(1.32)*	6.10 *(.83)*	2	.258	.003	.773
VLF %	4.75 *(2.87)*	5.93 *(4.38)*	5.82 *(3.18)*	2	1.708	.023	.185
LF %	52.42 *(17.89)*	54.07 *(16.52)*	56.80 *(15.09)*	2	.577	.008	.563
HF %	42.79 *(18.79)*	39.95 *(17.39)*	37.34 *(15.85)*	2	.885	.012	.415
LF n.u.	55.22 *(19.09)*	57.68 *(17.99)*	60.45 *(16.26)*	2	.742	.010	.478
Tot. pow.	1608.92 *(1501.23)*	1664.35 *(1547.81)*	1711.12 *(1568.20)*	2	.042	.001	.959

Note. HRV = Heart Rate Variability, *ASD* = Autism spectrum disorders, *n* = number, *M* = mean, *SD* = Standard Deviation, *df* = degrees of freedom, *F*=F-Test, η_p^2 = partial eta squared, *$p < .05$. Means with differing subscripts within rows are significantly different based on Bonferroni-corrected post hoc comparisons. *PNS* = parasympathetic nervous system index, *SNS* = sympathetic nervous system index, *SDNN* = standard deviation of R–R intervals in ms., *Mean HR* = mean heart rate in beats per minute, *RMSSD* = square root of the mean squared differences between successive R–R intervals in ms., *VLF* = absolute very low frequency power in ms^2, *LF* = absolute low frequency power in ms^2, *HF* = absolute high frequency power in ms^2, *VLF log.* = logarithmic very low frequency power, *LF log.* = logarithmic low frequency power, *HF log.* = logarithmic high frequency power, *VLF %* = very low frequency power in percent, *LF %* = low frequency power in percent, *HF %* = high frequency power in percent, *LF n.u.* = low frequency power in normalised units, *Tot. pow.* = Total power in ms^2.

Interestingly, the AQ scores were positively correlated with the SNS index ($r = 0.188$, $p = .021$, $R^2 = 0.035$, $n = 150$) and negatively correlated with the PNS index ($r = -0.202$, $p = .013$, $R^2 = 0.041$, $n = 150$). Moreover, the AQ scores were positively correlated with the mean HR ($r = 0.188$, $p = .021$, $R^2 = 0.035$ $n = 150$) and negatively correlated with RMSSD ($r = -0.165$, $p = .044$, $R^2 = 0.027$, $n = 150$) and VLF pow. log. ($r = -0.197$, $p = .015$, $R^2 = 0.039$, $n = 150$).

3.5. ROC analyses of the diagnostic tools compared to biological parameters

Since the AQ scores unsurprisingly proved to be a reliable measure for autistic traits, we calculated its sensitivity and specificity to discriminate between patients with ASD and all other patients (pooled OD/ND) using a ROC analysis. As can be seen in Fig. 1 the area under the curve (AUC) was 0.697 (95% CI = 0.613-0.781), indicating an acceptable discriminative power. In our sample of 55 patients diagnosed with ASD, 47 (85.5%) scored 32 or higher in the AQ questionnaire, while 54 out of 96 subjects not diagnosed with ASD (56.3%) scored 32 or higher ($\chi^2(1, n = 151) = 13.465$, $p < .001$).

In comparison, the ROC curve based on a regression analysis including all self-reported measures and third-party assessments as used in the clinical diagnostic procedure revealed an AUC of 0.856 (95% CI = 0.795-0.917). As expected due to its computational basis, this indicates an excellent power of discrimination (ASD vs. pooled OD/ND).

However, the ROC curve based on a regression analysis including only biological measures like sex, age, and all reported HRV parameters revealed an AUC of 0.736 (95% CI = 0.652-0.820). Interestingly, this still indicates an acceptable power of discrimination.

4. Discussion

The main results of the HRV parameters analysed showed significant differences between patients with ASD (ASD) and patients not meeting the criteria for a psychiatric diagnosis (ND). However, the differences between patients with ASD and patients with other psychiatric diagnosis (OD) did not reach significance. Specifically, both the SNS index indicating higher sympathetic activation, and the mean HR were significantly higher for ASD than for ND but did not significantly differ between ASD and OD. Even though the differences between ASD and OD, and OD and ND were not significant, the pattern of the SNS index and the mean HR values was in line with our first hypothesis: ASD had higher values than OD, and OD had higher values than ND. This was for example also the case for the stress index commonly associated with sympathetic activation. Additionally, a reversed pattern was found for HRV parameters commonly associated with parasympathetic activation such as the PNS index and the RMSSD: ASD had lower values than OD, and OD had lower values than ND.

Likewise, the AQ was positively correlated with the SNS index and negatively with the PNS index, which was partially in line with our second hypothesis. These results indicate that autistic behaviour as assessed by the AQ score might be associated with a higher sympathetic and a lower parasympathetic activation as assessed by the HRV.

In sum, these findings are consistent with previous research on HRV in adults with ASD indicating an overall dysregulation in resting autonomic activity (Cheng et al., 2020; Thapa et al., 2019). While previous research compared ASD populations to healthy control groups, our study extends the pre-existing evidence for an altered autonomic activity in ASD to more heterogenous clinical populations. Namely, we found significant differences in HRV between patients with ASD and patients not meeting the criteria for a psychiatric diagnosis, but still attending an outpatient clinic for a specialised assessment. And even though not statistically significant, the pattern of results might nonetheless indicate differences in sympathetic and parasympathetic activity between ASD and OD, as well as OD and ND. Further investigation is therefore required.

If confirmed, important implications for clinical settings arise. A widely accessible, non-invasive and inexpensive biomarker such as the ECG-derived HRV would allow for a much needed, time-efficient and reliable diagnostic assessment. Even though other psychiatric conditions are also associated with altered ANS activity, there might be a specific alteration in ASD allowing for differentiation. Considering the lack of flexibility and adaptability as a core symptom of ASD and the HRV as an index for flexibility and adaptability to stressors, this is arguably also in line with theoretical considerations.

What is more, the ROC analyses revealed an acceptable

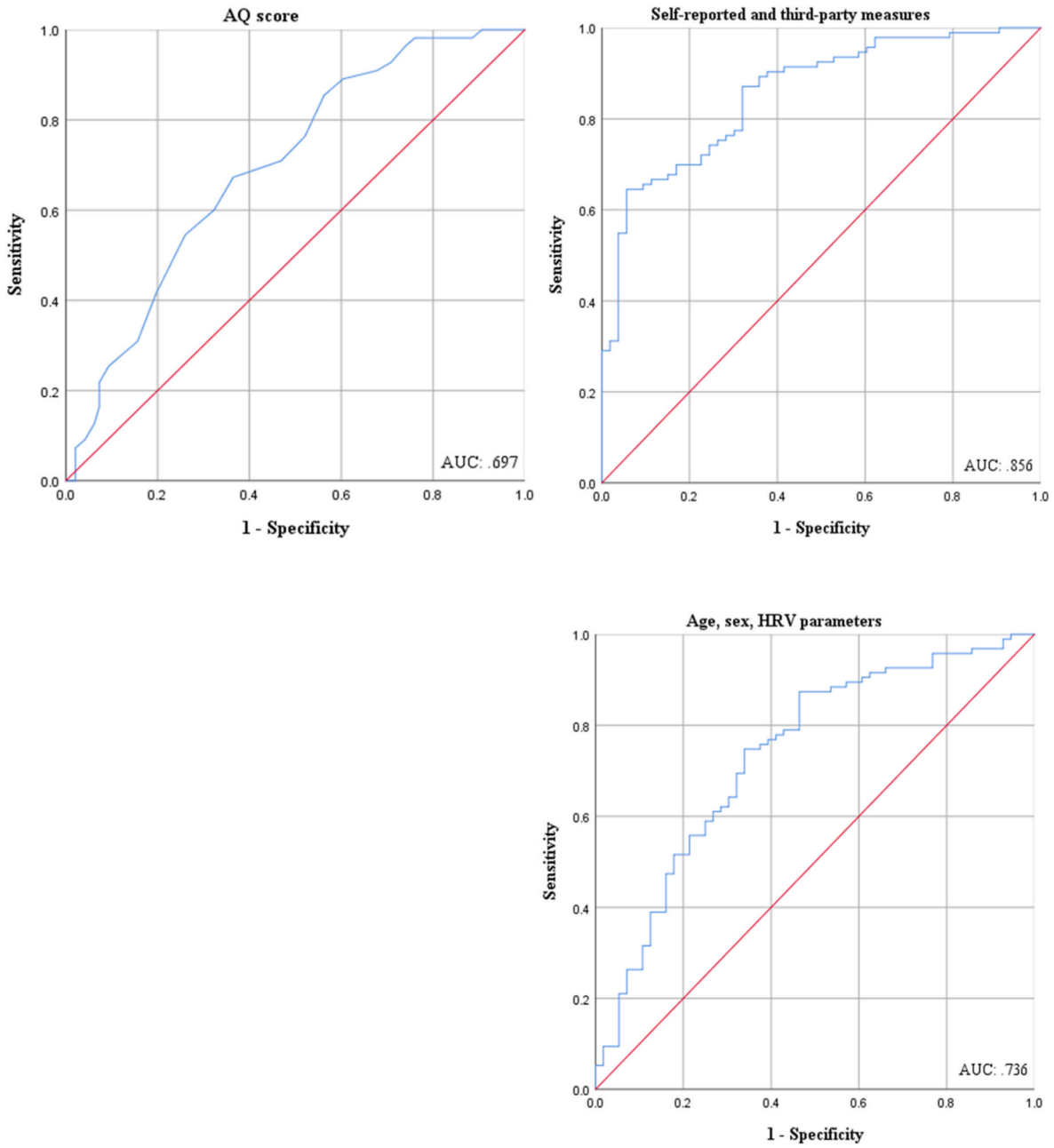

Fig. 1. Comparison of receiver operating characteristic (ROC) curves for Autism Quotient (AQ) scores (AUC = 0.697), self-reported and third-party assessments (AUC = 0.856), and biological parameters (AUC = 0.736).

discriminative power of the self-reported AQ score, which to date was only outperformed by a considerable set of self-reported questionnaires and third-party assessments provided by experienced clinicians. Interestingly, the ROC analysis for biological markers only, including sex, age, and HRV, revealed a slightly better discriminative power than the AQ score. In addition to time and cost efficiency, these findings highlight the clinical advantages of biological markers in ASD diagnostics. Healthcare professionals not specialised in ASD could for example contribute to reduce the waiting time for an ASD assessment by providing preliminary diagnostic assessments based on biological markers, ideally combined with self-reported questionnaires such as the AQ.

However, the study design holds several limitations. First, the group of patients with other psychiatric diagnoses was very heterogenous, including patients with personality disorders, affective disorders, psychotic disorders, or obsessive-compulsive disorder. This reflects the clinical challenges met during diagnostic assessments, which is the reason why they were merged into one group. However, previous findings have shown different psychiatric disorders to have different alterations of HRV parameters (Sikorska et al., 2018), which might have been blurred out by combining them into one group. Unfortunately, the small numbers of the OD subgroups did not allow for separate analyses.

Second, both groups of patients with ASD and other psychiatric diagnoses showed psychiatric comorbidities and psychotropic medication use. In order to identify the effect of other psychiatric conditions on the HRV, it might be useful to categorise patients with ASD into subgroups with different comorbidities. In our case the small numbers did not allow for an analysis of the comorbidities found in the ASD group. Moreover, we only controlled for stimulants and beta blocker intake, whose impact on the HRV are well investigated. However, the majority of the ASD patients (67.9%) did not have any comorbidities, and the psychotropic medication use, as well as the diagnoses of the OD group, were mostly comparable to the medication use and comorbidities of the ASD group.

Furthermore, the strong effect of tricyclic antidepressants on HRV and HR (van Zyl et al., 2008) could be ruled out, since they were not used in our sample.

Third, it remains unclear if the ECG/EEG recording itself accounted for differences between the groups. With the chosen setting this question cannot be addressed reliably. However, if an individual of the OD or ND group shows no signs of elevated sympathetic activity but an ASD patient does due to the arousal, this still constitutes a difference between the groups.

Moreover, we used the total length of the ECG recording comprising 15 min in order to provide sufficient data length for the analysis of different parameters, including VLF power. Since the literature reports recordings between 2 and 24 min of resting state recording, comparisons with other studies should be done with caution.

Furthermore, the diagnostic outcome and therefore our groups, were strongly influenced by the measures later used for correlative analyses and modelling. Lastly, due to its explorative nature, no corrections for multiple correlations were applied. Hence, the results should be interpreted with caution. Replications on similar but independent samples will be needed.

Statement of ethics

This study protocol was reviewed and approved by the Ethic Commission of the Kanton Zurich (Kantonale Ethikkommission Zürich), approval number: 2019–01616. The study was conducted according to the principles of the Declaration of Helsinki (2008). All participants were carefully informed about the study and informed written consent was obtained.

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Author contributions

T.V. and S.O. conceptualised the study, collected the data and constructed the data-set. T.V. carried out the statistical analysis, which was refined by A.R., A.B, and S.O. The first draft of the report was prepared by T.V., A.R., and A.B. and then revised by M.R., B.K., and S.O. All authors reviewed and contributed to the final draft and approved the final version for publication.

Data availability

Following the data policy of the University Hospital of Zurich, the data that supports the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy restrictions.

Declaration of competing interest

None declared.

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