Serum drug levels and medication adherence in heart failure: A comparative cohort analysis

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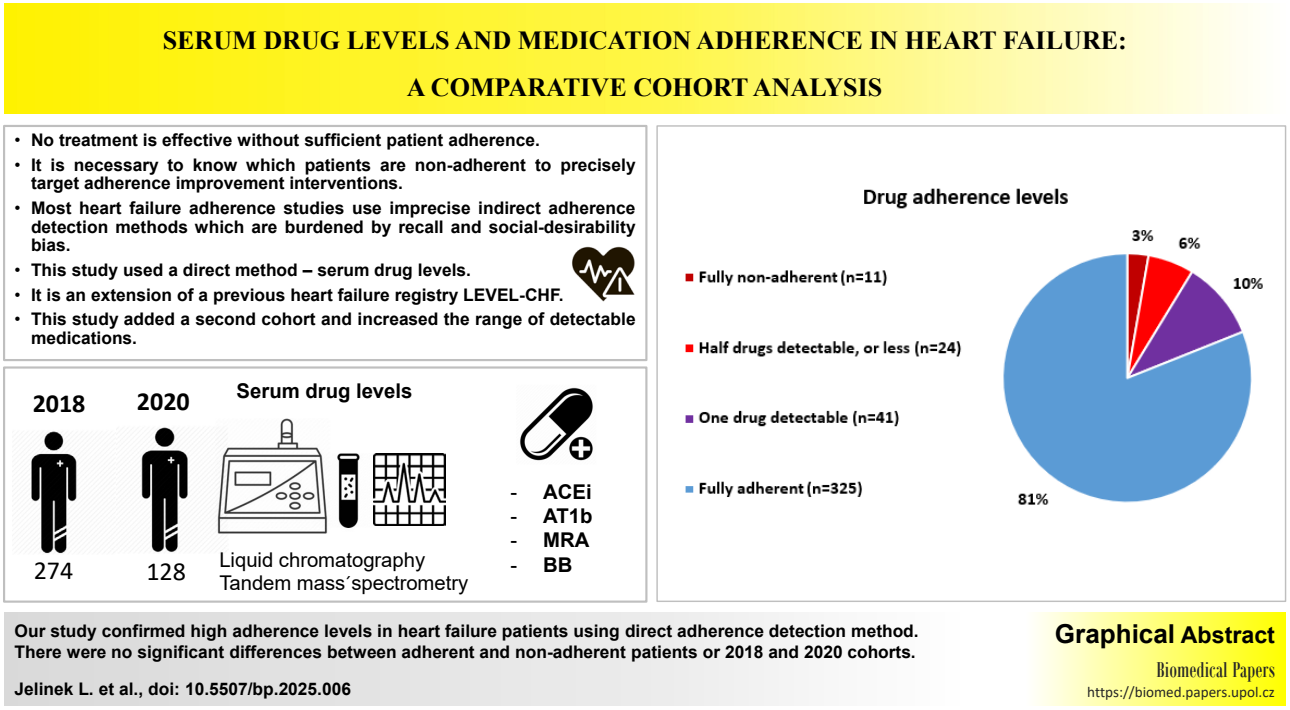
Objective. To determine changes in medication adherence in two cohorts of heart failure patients differing by year of data collection and using a direct method of adherence detection – serum drug level testing.
Methods. We added a second cohort of patients to a prospective monocentric registry of chronic heart failure patients (LEVEL-CHF registry). The two cohorts share the same inclusion criteria but differ by the year of enrolment (2018 and 2020). Stable patients with heart failure with reduced ejection fraction were enrolled in a specialized university hospital center.
Results. We included 402 records of 366 individual patients, 274 in 2018 and 128 in 2020. 36 patients were enrolled in both cohorts. Of the total 81% of patients were fully adherent, and 19% were non-adherent to a varying degree. Between 2018 and 2020 there was a statistically significant increase in BMI (P=0.047) and fasting glycemia (P=0.009). Patients in the 2020 cohort were less adherent than those in the 2018 cohort (P<0.01). Patients in the two cohorts had similarly severe heart failure and did not substantially differ in NYHA class. There were no statistically significant differences between adherent and non-adherent patients after adjusting for multiple comparisons.
Conclusions. In this comparison, most patients were fully adherent to all their medication and very few were non-adherent to multiple medications. We found no clinically relevant differences between adherent and non-adherent patients. Serum drug level testing is an effective method of adherence testing in clinical practice.



Key words: heart failure, adherence, compliance, serum drug levels, pharmacotherapy

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INTRODUCTION

Background

Background on heart failure and its global prevalence

Medicine in the 21st century is faced with a worldwide increase in the prevalence of chronic diseases[1]. Among these the prevalence of chronic heart failure in the global West is around 1–2% of the general population, with a significant increase after the age of 65, when heart failure becomes the leading cause of hospital admissions[2]. Although the age-adjusted incidence of heart failure is declining worldwide, the overall number of cases continues to rise due to improved survival rates[3,4]. Despite advances in treatment, mortality remains very high[5].

Financial costs

In addition to the fundamental importance of the disease to patients and their communities, heart failure is a significant economic burden on public and private health budgets. The treatment of cardiovascular diseases costs 155 billion annually in the European Union, representing 11% of total health costs[6]. Heart failure is the terminal stage of virtually all cardiovascular diseases and its treatment represents about 2% of the total costs[7].

Importance of heart failure medication

In recent years, there has been a significant shift in the treatment of heart failure due to the inclusion of new drug groups used in treatment, namely ARNI (Angiotensin Receptor Neprilysin Inhibitor) and Sodium Glucose Transporter 2 (SGLT2) inhibitors[8,9]. For any treatment to be effective and function as intended by the physician, it is essential that the patient adheres to the prescribed treatment regimen.

Importance of medication adherence in heart failure patients

Adherence to medication in heart failure is a major clinical problem and a concern to be addressed in treatment. Medication non-adherence to major heart failure medication groups is associated with an increase in all-cause mortality and heart failure hospitalizations[10]. There are effective methods for improving adherence, mainly targeted education, drug regimen simplification, and various forms of monitoring[11]. In a meta-analysis of 55 studies involving 15,016 patients with chronic heart failure, Unverzagt et al. found that interventions to improve adherence were effective in 10% of patients and led to a 2% improvement in long-term overall mortality and a 10% reduction in hospital admissions for heart failure[11].

The need for the study

To target interventions to individual patients, we need to know who is non-adherent or at risk of non-adherence. The reported adherence to medication for heart failure varies quite substantially based on the population studied and the method of detection, ranging between 10 and 98% (ref.[12]). Adherence detection methods are divided into direct and indirect based on the extent to which they rely on information self-reported by individual patients. There are pros and cons for the various methods which are suitable for several different clinical and research situations. The main advantage of direct methods, such as serum drug level testing, is that they measure the objective presence of a molecule in the serum and are therefore not influenced by the patient or physician[13]. The disadvantage is the cost and technical complexity of the method.

Aims of the study

The primary objective of this study was to determine the level of adherence to medication in a defined clinical population of patients with chronic heart failure followed at a tertiary care hospital using a direct method of adherence detection – by measurement of serum drug levels.

Secondary objectives were to compare the cohorts of patients enrolled in 2018 and 2020 in clinical parameters and adherence, to compare adherent and non-adherent patients overall, and to determine if any trends and correlations in clinical parameters are associated with medication adherence.

METHODS

Study design

This was a monocentric prospective study on a stable heart failure patients. The study included two cohorts separated by a follow-up time of 2 years. Data from the first cohort have already been published previously (LEVEL-CHF registry) (ref.[14]). We named the overall registry as LEVEL-CHF ext. to differentiate the studies.

Data collection

Data for this study were obtained during regular outpatient follow-ups at the tertiary care hospital. Data for the first cohort were collected between January and June 2018 (274 subjects). Data for the second cohort were collected between January and February 2020 (128 people). In the second cohort, 36 patients who had participated in the first phase 2 years earlier were re-enrolled. A total of 427 records were screened, 16 patients did not fulfill the diagnostic criteria for heart failure according to the guidelines and 9 patients refused to give informed consent for inclusion in the study. Overall, 402 records of 366 individual patients were included.

The original aim was to recruit a similar number of patients in the second cohort as in the first, but recruitment was terminated by the COVID-19 pandemic, which canceled outpatient follow-up. Participation in the study was offered to all patients at the center who presented for

a regular check-up during the period and had not had a change in medication for at least 1 month.

The clinical characteristics of patients were regularly monitored during the doctor's visit. For each patient, demographic data such as age, sex, weight, height, body-mass index (BMI), and clinical parameters including the New York Heart Association (NYHA) classification, heart rate, and systolic and diastolic blood pressure were extracted from the electronic hospital record of the check-up. The ejection fraction was determined by routine echocardiography. Periodic blood tests were used to determine laboratory values of a range of parameters, from basic electrolytes to specific cardiac markers such as NT-proBNP (Table S1 in supplementary data). Further, specifically for this study, blood tests included the collection of serum levels of heart failure medications and other drugs detectable by our methods. Both clinical check-ups and laboratory tests were done on the same day. Patients received information about the sampling of drug levels and informed consent just before the blood was drawn. In this way, they were unable to prepare for the test in advance. However, "white coat adherence", where the patient takes the medication just before the doctor's visit and is nonadherent the rest of the time, cannot be ruled out.

The designation of a patient as adherent was used if they had detectable medications of all basic classes of guidelines recommended heart failure treatment – renin-angiotensin-aldosterone system (RAAS) blockers, beta-blockers, and mineralocorticoid receptor antagonists (MRA) if they were prescribed to them. Testing for SGLT2 inhibitors was not available in our laboratory during the study enrolment period. If the level was not detectable for at least one of these classes, the patient was designated as non-adherent. Although other drug groups were measured, due to the variability of drug regimens, different drug indications, and unclear impact on the prognosis of heart failure patients, these drugs were not used in the comparison of the cohorts of adherent and nonadherent patients. See Table 1 for a full list of detectable drug groups and individual drugs.

Laboratory analysis of serum drug levels

Analysis of beta-blockers

A UHPLC UltiMate 3000 RSLC System (Dionex, Sunnyvale, CA, USA) equipped with a UHR-TOF Maxis Impact HD (Bruker Daltonics, Billeric, MA, USA) was used for the analyses. Reversed phase column Acclaim RS 120 (Thermo Fisher Scientific, Waltham, MA, USA) at a temperature of 40 °C was used for the beta-blocker's separation. Gradient elution at a flow rate of 0.5 mL/min was applied. Positive electrospray ionization in the positive ionization mode was applied using a UHR-TOF mass spectrometer.

Further details of sample preparation are provided in the Online Supplement.

Analysis of the rest of the drug groups

The prepared samples were analyzed using high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). LC-MS/MS analysis was performed on HPLC instrument UltiMate 3000 RS (Dionex, Sunnyvale, CA, USA) using Acquity BEH C18 column (1.7 µm, 2.1 × 50 mm, Waters, Milford, MA) and triple quadrupole mass spectrometer Triple Quad 6500 (Sciex, Framingham, MA, USA). Detection was carried out using the positive/negative electrospray ionization technique and multiple reaction monitoring mode.

Further details of sample preparation are provided in the Online Supplement.

Statistical Analysis

Data preparation was performed using R programming language[15]. Visualization was performed using the ggplot2 package[16]. Comparisons between pairs of groups were made with the Wilcoxon rank-sum test for continuous variables and the Chi-squared test for binary variables.

When false positive results of a test would undermine our claims, *P*-values were adjusted for multiple testing following the Benjamini-Hochberg method[17]. Notably, we made no multiple testing correction in Table 2 and Table 3 as any difference between the cohorts is a potential problem for our conclusions and thus a bigger risk lies in false negatives and a multiple testing correction would obfuscate the issue.

Table 1. Full list of detectable drug groups and individual drugs.

	Drug group	Individual drugs			
RAAS blockers	ACE inhibitors	Perindopril	Ramipril	Trandolapril	
	AT1 receptor blockers	Telmisartan	Candesartan	Losartan	Valsartan
	ARNI	Sacubitril/Valsartan			
Diuretics	MRA and potassium sparring diuretics	Spironolactone	Eplerenone	Amiloride	
	Loop diuretics	Furosemide			
	Thiazide and Thiazide-like diuretics	Indapamide	Chlorthalidone	Hydrochlorothiazide	
Others	Beta-blockers	Bisoprolol	Metoprolol	Carvedilol	Nebivolol
	Ca blockers	Nitrendipine	Amlodipine	Lercanidipine	
	Alpha-blockers	Doxazosin			
	Cardiac glycoside	Digoxin			
	Statins	Atorvastatin	Rosuvastatin		

Ethical considerations

Data recruitment, study design, and the informed consent form were approved by the local ethics committee under reference number 175/17. All participants signed informed consent and were over 18 years of age. The study design was in accordance with the latest Declaration of Helsinki.

RESULTS

Comparison of the 2018 and 2020 cohorts

In addition to the description of the characteristics of the whole set, we compared the clinical and laboratory parameters of patient cohorts from both years. See Table 2 for the results. Most clinical parameters were substantially the same between cohorts. There is a small, but statistically significant, increase in BMI and fasting glycemia. Patients in both cohorts had similarly severe heart failure and did not differ in NYHA class. Because physicians often use transient NYHA classes (e.g., NYHA 2-3), we retained these intermediate grades in the analysis to avoid loss of information.

See Table 3 for a comparison of the percentage representation of each drug group. There was a significant replacement of angiotensin-converting enzyme (ACE) inhibitors by ARNIs (between 2018 and 2020). Other drug classes proportions remained relatively stable, see Table 3. Drug groups not detectable by our current methods were not included in the analysis. Data were collected before the massive expansion of gliflozins and their promotion to the first-line choice of therapy for heart failure. In addition, they were not detectable by our methods and are therefore not included in the analysis. As we analyzed a patient population from routine clinical practice, the individual drug combinations varied considerably from patient to patient. For visualization of the combinations of different drug groups, see Fig. 1 and Fig. 2. The vast majority of patients were taking the guidelines-recommended combinations of drugs positively affecting prognosis, eventually with the addition of furosemide.

36 patients participated in both cohorts thanks to long-term follow-up at the center. Their persistence (adherence over time) did not change substantially. There were both shifts from adherent to non-adherent and vice versa, see Table 4.

Table 2. Comparison of clinical and laboratory parameters of the 2018 and 2020 cohorts and both together.

Characteristic	**(Both cohorts)** n = 402[1]	**2018**, n = 274[1]	**2020**, n = 128[1]	*P*[2]
Sex				0.6
Female	101 (25%)	66 (24%)	35 (27%)	
Male	301 (75%)	208 (76%)	93 (73%)	
Weight	90 (±19)	88 (±18)	92 (±21)	0.14
Height	174 (±9)	174 (±8)	174 (±10)	0.7
BMI	29.5 (±5.5)	29.1 (±5.4)	30.4 (±5.5)	0.047
Heart rate	71 (±13)	71 (±13)	72 (±14)	0.7
Systolic blood pressure	128 (±19)	128 (±19)	128 (±21)	0.9
Diastolic blood pressure	78 (±11)	79 (±11)	78 (±12)	0.6
Diabetes	128 (32%)	80 (29%)	48 (38%)	0.13
NYHA class				0.2
1	72 (18%)	51 (19%)	21 (16%)	
1-2	57 (14%)	35 (13%)	22 (17%)	
2	168 (42%)	125 (46%)	43 (34%)	
2-3	44 (11%)	25 (9.1%)	19 (15%)	
3	56 (14%)	35 (13%)	21 (16%)	
3-4	4 (1.0%)	2 (0.7%)	2 (1.6%)	
4	1 (0.2%)	1 (0.4%)	0 (0%)	
Left ventricle ejection fraction	35 (±11)	35 (±11)	36 (±10)	0.2
Na (mmol/L)	139.69 (±2.52)	139.61 (±2.46)	139.88 (±2.64)	0.2
K (mmol/L)	4.49 (±0.40)	4.48 (±0.41)	4.51 (±0.39)	0.12
Cl (mmol/L)	101.8 (±3.4)	101.9 (±3.4)	101.6 (±3.3)	0.5
Urea (mmol/L)	7.4 (±4.2)	7.3 (±3.7)	7.8 (±5.0)	0.6
Creatinine (mmol/L)	109 (±69)	111 (±78)	105 (±42)	0.6
Glycaemia (mmol/L)	6.86 (±2.29)	6.69 (±2.11)	7.23 (±2.60)	0.009
NT-proBNP (mg/L)	1537 (±3051)	1638 (±3406)	1324 (±2.105)	0.6
GFR (mL/min/1.73m^2)	1.12 (±0.38)	1.13 (±0.38)	1.12 (±0.39)	0.9
HbA1c in diabetics (mmol/mol)	47 (±12)	NA (±NA)	47 (±12)	

P-values are displayed for comparison between cohorts 2018 and 2020. Clinical parameters of 2018 cohort are adapted from previous publication, LEVEL-CHF registry[14].

[1]n (%); Mean (±SD), [2]Pearson's Chi-squared test; Wilcoxon rank sum test.

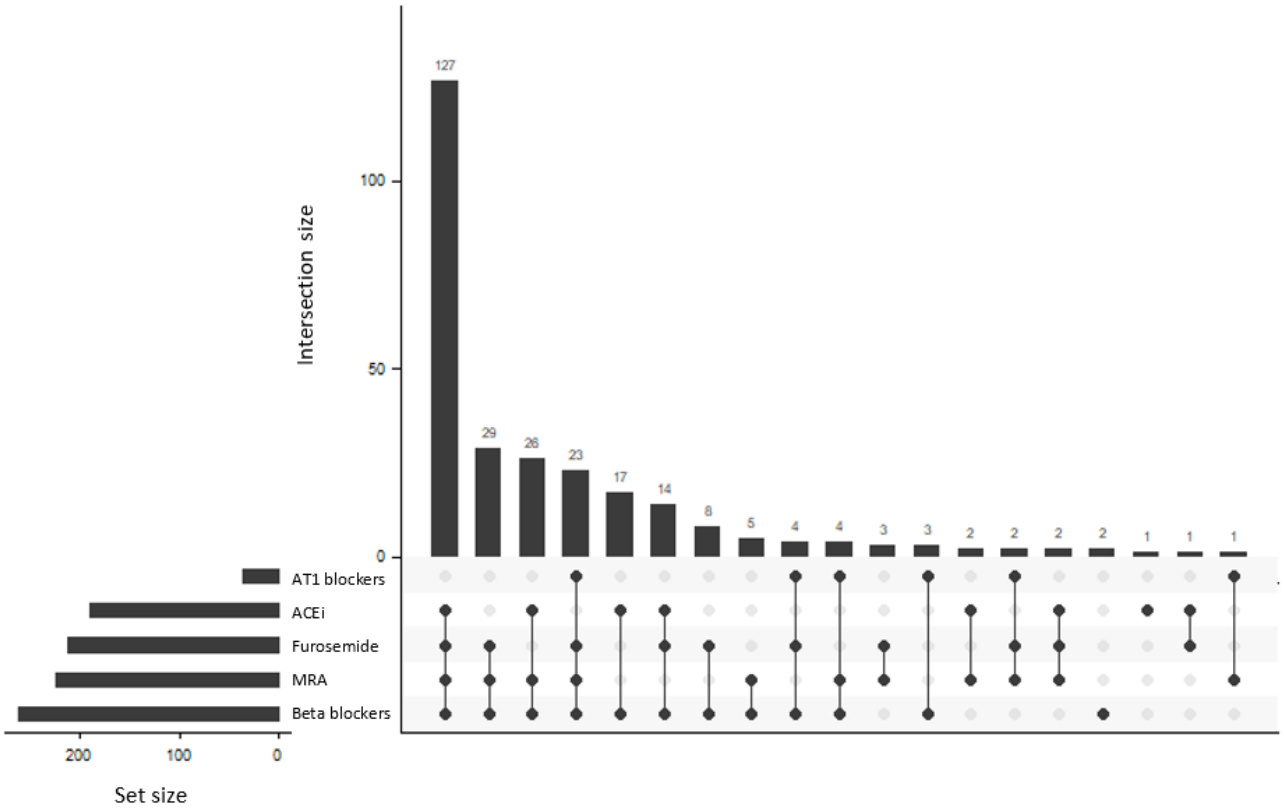

Fig. 1. Different drug groups combinations in the 2018 cohort.

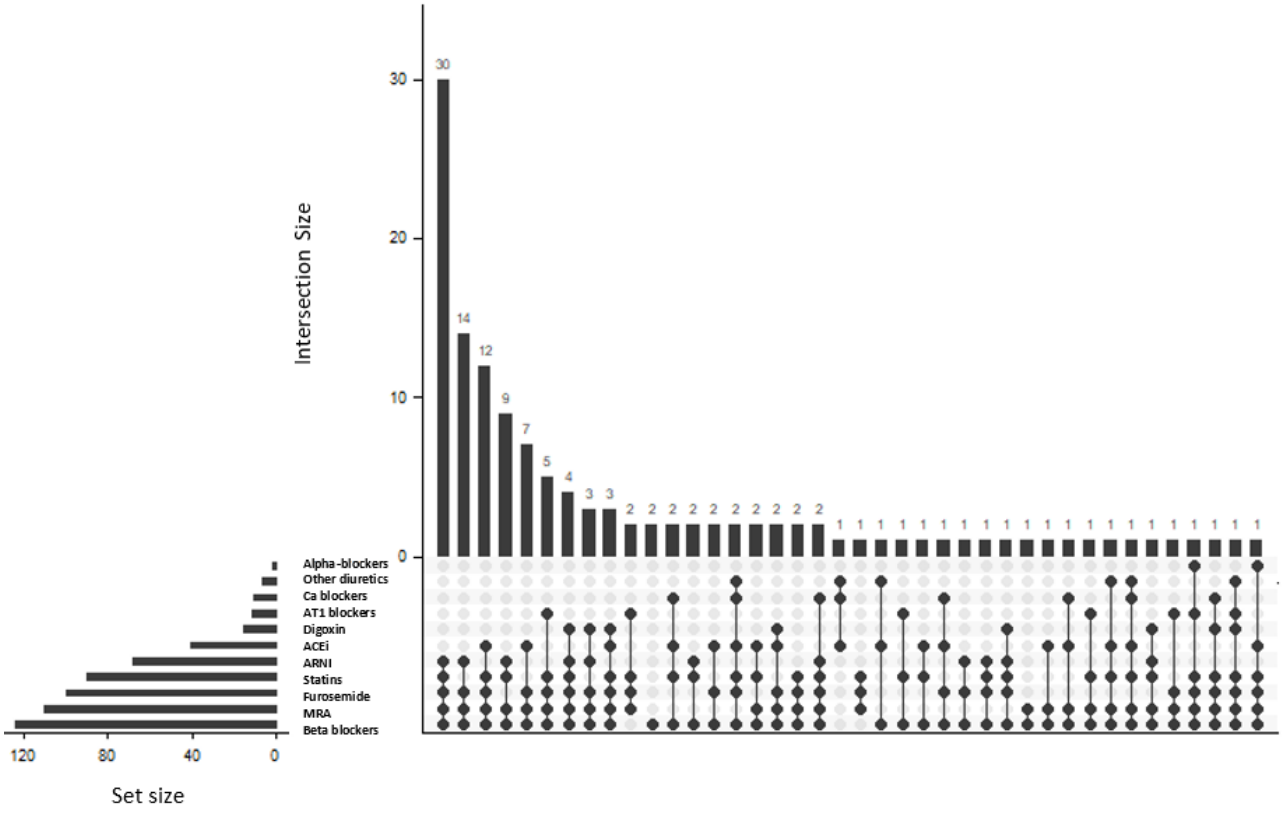

Fig. 2. Different drug groups combinations in the 2020 cohort.

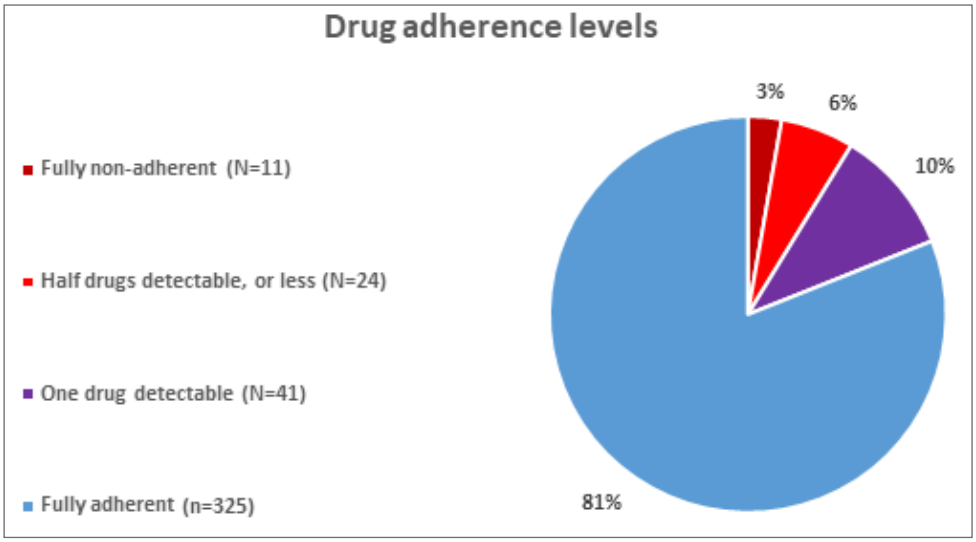

Fig. 3. Drug adherence levels.

Table 3. Comparison of the percentage representation of individual drug groups.

Drug Group	2018, n = 274[1]	2020, n = 128[1]	*P*[2]
ACE inhibitors	190 (70%)	41 (32%)	<0.001
AT1 blockers	37 (14%)	12 (9.4%)	0.3
Furosemide	213 (78%)	100 (78%)	>0.9
MR antagonists	224 (82%)	110 (86%)	0.4
Beta-blockers	262 (96%)	124 (97%)	0.7
Other diuretics	0 (NA%)	7 (5.5%)	
ARNI	0 (NA%)	68 (53%)	
Calcium channel blockers	0 (NA%)	11 (8.6%)	
Statins	0 (NA%)	90 (70%)	
Alpha-blockers	0 (NA%)	2 (1.6%)	
Digoxin	0 (NA%)	16 (13%)	

[1]n (%); Mean (±SD), [2]Pearson's Chi-squared test; Wilcoxon rank sum test.

Comparison of adherent and non-adherent patients

For the analysis of the adherent and non-adherent patients, we examined only adherence to RAAS blockers (ACE inhibitors, AT1 blockers, and ARNI), beta-blockers, and MRAs, because other drug groups were not detectable in the 2018 cohort. Other measured drug groups were excluded (from the 2020 cohort). Further, we excluded a single patient, who had not had any measurable medication apart from furosemide. Of the total, 81% of patients were fully adherent and 19% were non-adherent at varying levels. In our study cohort, most patients were fully adherent to all their medications and very few were non-adherent to multiple medications. See Fig. 3 for the distribution of the patient population according to adherence levels.

Further, we observed noticeable differences between cohorts. Patients from the 2020 cohort were less adherent than those from the 2018 cohort. For detailed data comparing adherence levels, see Table 5.

Next, we compared adherent and non-adherent patients with each other. After adjustment, there were no

Table 4. Changes in adherence for the same patients enrolled in 2018 and 2020.

Drug class	Change in adherence	n
ACEi	Become adherent	0
	Identical	6
	Become non-adherent	2
Beta-blockers	Become adherent	1
	Identical	29
	Become non-adherent	1
MRA	Become adherent	3
	Identical	18
	Become non-adherent	4

Table 5. Drug adherence levels comparison between the 2018 and the 2020 cohort.

Adherence Levels	2018	2020
Fully non-adherent	3.30% (n=9)	1.6% (n=2)
Half of drugs or less detectable	5.86% (n=16)	6.2% (n=16)
One drug undetectable	6.59% (n=18)	18.0% (n=23)
Fully adherent	84.25% (n=230)	74.2% (n=95)

The differences are statistically significant with Chi-squared *P*-values of $P<0.01$.

Table 6. Comparison of clinical and laboratory parameters of adherent and non-adherent patients.

Characteristic	Fully adherent, n = 325[1]	Non-adherent, n = 76[1]	*P*[2]	*P* adjusted[3]
Sex			0.9	>0.9
Female	83 (26%)	18 (24%)		
Male	242 (74%)	58 (76%)		
Age	63 (±13)	62 (±13)	0.4	0.7
Weight	89 (±19)	90 (±20)	>0.9	>0.9
Height	174 (±9)	174 (±10)	>0.9	>0.9
BMI	29.5 (±5.4)	29.8 (±5.9)	0.8	>0.9
Heart rate	71 (±13)	72 (±14)	0.9	>0.9
Diabetes	101 (31%)	27 (36%)	0.6	0.9
Systolic blood pressure	127 (±19)	134 (±22)	0.005	0.1
Diastolic blood pressure	78 (±11)	81 (±12)	0.006	0.1
NYHA			0.2	0.5
1	60 (18%)	12 (16%)		
1–2	45 (14%)	12 (16%)		
2	135 (42%)	32 (42%)		
2–3	36 (11%)	8 (11%)		
3	47 (14%)	9 (12%)		
3–4	1 (0.3%)	3 (3.9%)		
4	1 (0.3%)	0 (0%)		
Left ventricle ejection fraction	35 (±11)	34 (±11)	0.3	0.6
Na (mmol/L)	139.61 (±2.52)	140.08 (±2.50)	0.052	0.3
K (mmol/L)	4.51 (±0.42)	4.41 (±0.34)	0.030	0.3
Cl (mmol/L)	101.7 (±3.4)	102.3 (±3.1)	0.10	0.4
Urea (mmol/L)	7.6 (±4.4)	6.8 (±3.1)	0.14	0.4
Creatinine (mmol/L)	110 (±70)	104 (±62)	0.2	0.5
Glycaemia	6.72 (±1.99)	7.49 (±3.23)	0.12	0.4
NT-proBNP (mg/L)	1559 (±3124)	1410 (±2737)	0.8	>0.9
GFR (mL/min/1.73m^2)	1.12 (±0.38)	1.17 (±0.36)	0.3	0.6
HbA1c in diabetics (mmol/mol)	48 (±12)	43 (±16)	0.6	0.9
ACE inhibitors	190 (59%)	41 (54%)	0.5	0.9
AT1 blockers	36 (11%)	13 (17%)	0.2	0.5
Furosemide	251 (77%)	61 (80%)	0.7	>0.9
MR antagonists	267 (82%)	66 (87%)	0.4	0.7
Beta-blockers	312 (96%)	74 (97%)	0.8	>0.9
Other diuretics	6 (6.3%)	1 (3.0%)	0.8	>0.9
ARNI	53 (56%)	15 (45%)	0.4	0.7
Calcium channel blockers	9 (9.5%)	2 (6.1%)	0.8	>0.9
Statins	62 (65%)	28 (85%)	0.057	0.3
Alpha-blockers	0 (0%)	2 (6.1%)	0.11	0.4
Digoxin	12 (13%)	4 (12%)	>0.9	>0.9

[1]Mean (±SD); n (%), [2]Wilcoxon rank sum test; Pearson's Chi-squared test, [3]*P* after adjustment with the Benjamini-Hochberg method controlling for a false discovery rate at 5%.

statistically significant differences between the groups. Some weak evidence for differences in blood pressure (systolic and diastolic) and potassium levels is present. See Table 6 for a detailed comparison.

DISCUSSION

Comparison of the 2018 and 2020 cohorts

This was a study of two cohorts of patients who were enrolled in the same manner but in different years. Compared to the 2018 cohort, we examined more drug types in 2020 due to advances in detection methods. We did not observe any truly significant differences in patient mix between 2018 and 2020. There was not even a statistically significant shift in functional capacity between cohorts assessed by the NYHA class. The cohorts also had similar medication prescription rates. There has been a significant increase in prescribing ARNIs instead of ACE inhibitors (and AT1 blockers) due to improved funding and the inclusion of ARNIs as first-choice drugs according to guidelines[18]. In general, in our study, there was a high percentage of guideline-recommended heart failure therapy, indicating good physician adherence to guidelines. Good physician' adherence to guidelines is associated with improved outcomes in HF (ref.[19]). Overall, 89% of patients were prescribed RAAS blockers, beta-blockers and MRAs. This is probably a result of the enrolment of patients in a specialized university hospital heart failure center.

One observable difference between the cohorts is a 1.3 kg/m^2 increase in BMI. The trend of increased BMI is consistent with the increase in the general European population[20]. In the heart failure patient population, this may not be a strictly negative message because the survival curve is U-shaped due to the obesity paradox. In a study of 47,531 patients with heart failure, Jones et al. showed that patients who were overweight (risk difference −4.1%) or obese in grades 1 and 2 (both risk difference −4.5%) had better survival than those who were normal weight, underweight patients had increased risk of all-cause death (risk difference 11.2%) (ref.[21]). Our finding of higher fasting glycemia in the 2020 cohort could be explained by weight gain. This is consistent with the observed 9 percentage point increase in diabetes prevalence. These days, diabetes screening is particularly useful in patients with heart failure due to the synergy in the treatment of both diseases by SGLT2 inhibitors[9].

The overall adherence of our patients was very high. The level of adherence in studies is highly variable depending on the method of detection and population selection. Adherence by measuring serum drug levels was used in the study by Pelouch et al. In 81 patients with chronic heart failure, adherence was 75%. Half of the patients had repeated collection during outpatient follow-ups, and the adherence rates gradually decreased to 71% and 66%, respectively[22]. In our study patients in 2020 (74.2%) had significantly lower levels of adherence compared to the 2018 cohort (84.25 %). This was mainly driven by patients who were adherent to all drugs except one. The difference is not explained by a difference in clinical parameters. On the other hand, the persistence of our patients was high in a small sample of patients with adherence measured in both 2018 and 2020. Reductions in population adherence, as opposed to reductions in persistence over time in specific patients, are not described in the corresponding literature. A large study by Ødegaard et al. on a population of 54,899 patients followed between 2014 and 2020 found no decrease in adherence in the same drug categorizations using the proportion of days covered (PDC) method[23].

Comparison of the adherent and the non-adherent patients

The data are from a real patient population, hence, there is a variety of drug combinations due to the combination of adverse effects and comorbidities. This considerable variability in medication makes it difficult to interpret the results and search for differences between patients. For example, loop diuretics decrease potassium and RAAS blockers and MRAs increase it, different patients on different combinations of these medications will have highly variable potassium levels and it is difficult to distinguish the effect of medication adherence. In patients with heart failure, monitoring potassium levels is an important clinical task because both hypokalemia and hyperkalemia are associated with a poor prognosis[24]. Despite the trend of difference, both adherent and non-adherent patients had normal mean serum potassium levels.

Strengths

The main strength of our study is the use of direct adherence measurement. The presence of the metabolite in serum cannot be distorted in any way. As of the date of publication, this is the largest sample of heart failure patients examined in this way. Investigation of serum drug levels is a common method in arterial hypertension but is not used nearly as much in heart failure despite the possibility of using the same technology due to the overlap of effective medication between the two diseases.

Another advantage was the repeated measurement of the population using the same method of patient recruitment. The only difference was the greater range of detectable agents due to advances in the method used.

Results between the 2018 and 2020 cohorts were substantially unchanged, indicating a high degree of replicability of results in the same population.

Limitations

The main limitations of our study include single adherence sampling due to the chosen method. Longitudinal as opposed to cross-sectional study design might give different data. Repeated sampling would significantly increase costs. We also anticipated that by sampling two cohorts of patients at the same center 2 years apart, there would inevitably be some overlap, and we would have a definite number of patients with 2 samplings. This did happen, but only in 36 cases because of the cessation of patient recruitment due to the covid 19 pandemic.

Another limitation is the low number of non-adherent patients; when designing the study, we assumed that there would be more non-adherent patients (due to experience with directly measured adherence in patients with arterial hypertension) and thus significant differences between adherent and non-adherent patients (if there are any) would be more detectable.

Further limitation is the use of a sample of patients with unequal drug regimens. Although we found no significant differences between adherent and non-adherent patients in percentage of medications, the variability in medication regimens was considerable between patients and made subsequent data analysis difficult.

Considering the currently used common heart failure drug regime, another limitation is a lack of a method to detect SGLT2 inhibitors, although, during the enrolment of patients, the prevalence of this drug was still very low.

Our patient sample also does not represent the general population of heart failure patients. These were patients followed up in a specialized center of a teaching hospital. Also, there was a higher representation of men than in the general patient population, which may have been due to the higher representation of ischemic etiology of heart failure, which is more common in men, and the non-inclusion of a different form of heart failure syndrome – heart failure with preserved ejection fraction, which is more common in women.

Recruitment to the study was completed before the main impact of the pandemic in the Czech Republic. However, despite this, limitations in access to medical care may have affected our results. It can be argued that patients with longer commutes to the university hospital may have presented less frequently for check-ups during the pandemic and thus shift our population towards those living in a major city, who could have different demographic parameters such as education or income.

Future perspectives

Given the general increase in the number of clinical trials, the wide variability in methods for determining adherence to drug regimens, and the multiple definitions of heart failure, comparing studies and drawing conclusions is likely to become increasingly difficult in the future.

It would be useful to establish definitions of medication adherence and make more use of direct detection methods that do not involve as much bias. A good definition of adherence and identification of less adherent populations may allow better targeting of interventions to improve adherence and thus save time and resources.

CONCLUSION

Our analysis provides insights into patient characteristics, medication utilization, and adherence patterns, which could be valuable for healthcare professionals to understand patient behavior and potentially improve treatment outcomes.

Comparison of the 2018 and the 2020 cohorts

Most clinical parameters between the two cohorts remained consistent. However, there was a small, statistically significant increase in BMI (Body Mass Index) and fasting glycemia. The severity of heart failure, as measured by the NYHA (New York Heart Association) class, was similar across both cohorts.

We observed a significant shift from the use of ACE inhibitors to ARNIs between 2018 and 2020. Other drug classes remained relatively stable, and most patients were on guideline-recommended drug combinations.

Adherence to RAAS (Renin-Angiotensin-Aldosterone System) blockers, beta-blockers, and MRAs (Mineralocorticoid Receptor Antagonists) were analyzed. The analysis revealed that 81% of the patients were fully adherent, while 19% were non-adherent to varying degrees. The 2020 cohort showed less adherence (74.2%) compared to the 2018 cohort (84.3%), which was statistically significant (P<0.01). Contrary to usual results medication persistence of our patients remained high.

Comparison of adherent and non-adherent patients

We cannot draw any firm conclusions from the comparison between adherent and non-adherent patients. There were very few non-adherent patients, and in general, patients varied widely in their drug regimens, so e.g., the metabolic effect would be biased by conflicting biological effects (e.g., for potassium levels).

ABBREVIATIONS

HFrEF, Heart Failure with reduced Ejection Fraction; HFpEF, Heart Failure with preserved Ejection Fraction; CHF, Chronic Heart Failure; ARNI, Angiotensin Receptor Neprilysin Inhibitor; ACEi, Angiotensin Converting Enzyme inhibitor; BB, beta-blocker; MRA, mineralocorticoid receptor antagonist; AT1, angiotensin 1; BMI, body mass index; SD, standard deviation; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; ALP, Alkaline Phosphatase; GGT, Gamma-glutamyl Transferase; MCV, Mean Cell Volume; MCH, Mean Cell Haemoglobin; MCHC, Mean Cell Haemoglobin Concentration; RDW, Red Cell Distribution Width; PLT, Platelet Count; MPV, Mean Platelet Volume; NT-proBNP, N-terminal pro B-type Natriuretic Peptide; CRP, C-reactive Protein; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; TSH, Thyroid Stimulating Hormone; aPTT, Activated Partial Thromboplastin Time; PT, Prothrombin Time; EF, Ejection Fraction; SDL, serum drug level; MPR, medication possession ratio; PDS, proportion of days covered; SGLT, Sodium-Glucose-Transporter.

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SUPPLEMENTARY DATA

Table S1. Standard follow-up blood tests.

Biochemistry	Serum sodium, potassium, chlorides, magnesium, urea, creatinine, uric acid, bilirubin, AST, ALT, ALP, GGT, NT-proBNP, CRP, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, free blood iron, ferritin, TSH, glucose, HbA1c
Complete blood count	leukocytes, erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelets, MPV, blood cells differential (relative and absolute)
Coagulation	PT, INR, aPTT

Laboratory analysis of serum drug levels

Analysis of beta-blockers

Serum samples for the determination of selected beta-blocker were prepared as follows, Blood samples were centrifugated at 3,000 rpm/min for 5 min, and separated serum samples were treated: (i) in case the determination of bisoprolol, carvedilol, and metoprolol 50 µL of serum was spiked with 5 µL of D7-metoprolol as internal standard (concentration 2 µg/mL). The mixture of serum with internal standard was precipitated by 150 µL of methanol and vortexed for 1 min. The serum sample was centrifuged at 3,000 rpm/min for 5 min and the supernatant was transferred to a vial with insert and injected into the LC-MS system. Injection volume was 5 µL; (ii) in case of the determination of nebivolol 50 µL of serum was spiked with 3 µL of D7-metoprolol as internal standard (concentration 2 µg/ml). The mixture of serum with internal standard was precipitated by 150 µL of methanol and vortexed for 1 min. The serum sample was centrifuged at 3,000 rpm/min for 5 min and the supernatant was transferred to a vial with insert and injected into the LC-MS system. The injection volume was 10 µL.

Analysis of the rest of the drug groups

Whole blood was collected in tubes containing anticoagulant tri-potassium salt of ethylenediaminetetraacetic acid (K3EDTA), and centrifuged (15 min, 1500 x *g*), plasma was removed and frozen at -20 °C. Before analysis, all samples were thawed at 4 C. Plasma (100 µL) was mixed with a mixture of deuterated internal standards (10 µL, 1000 ng/mL – perindopril- d4, perindoprilat- d4, ramipril-d5, ramiprilat-d5, spironolactone-d6 a telmisartan-d3 and precipitated by 0.3 mol/L $ZnSO_4$ in 70% methanol (200 µL). After vortex mixing and centrifugation (5 min, 14 500 × *g*) the supernatant was dried under a nitrogen stream at 40 °C. The residue was then reconstituted with mobile phase (100 µL, A/B, 9:1 (v/v)) and vortex mixed.

The prepared samples were analyzed using high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). LC-MS/MS analysis was performed on HPLC instrument UltiMate 3000 RS (Dionex, Sunnyvale, CA, USA) using Acquity BEH C18 column (1.7 µm, 2.1 × 50 mm, Waters, Milford, MA) and triple quadrupole mass spectrometer Triple Quad 6500 (Sciex, Framingham, MA, USA). Mobile phase A contained 28 mm ammonium formate buffer (pH 2.8) and mobile phase B 100% methanol. The gradient elution at a flow rate of 0.4 mL/min was as follows. It started at 5 % mobile phase B. After 0.1 min at initial conditions, it ramped to 90 % over 1.5 min, held at 90 % for 1.5 min, and then returned to 5 % B in 0.1 min with an analysis time of 3.7 min. The column was tempered at 50 °C, the injection volume was 1 µL and the autosampler temperature was 10 °C.

Detection was performed using positive/negative electrospray ionization technique and multiple reaction monitoring mode (Table XY). Both quadrupoles were set at unit resolution. The ion source parameters and gases were set as ion spray voltage: 5500/-4500 V; collision gas: 6 arb; curtain gas: 35 arb; both ion source gases: 40 arb and source temperature: 400°C. Declustering potentials (DP), entrance potentials (EP), collision energies (CE), and collision cell exit potentials (CXP) were optimized for each compound by the previous infusion of standards.

MS operation was conducted using Analyst® software 1.6.2 while data processing was carried out by SciexOS 2.0 (Sciex, Framingham, MA, USA).

Table S2. MRM analysis conditions.

Compound	Internal Standard	Polarity	Q1 (Da)	Q3 (Da)	Time (ms)	DP	EP	CE	CXP
Amiloride	Amlodipine-d4	Positive	229.9	171.0	30	96	10	25	16
Amlodipin	Amlodipine-d4	Positive	409.1	237.9	10	46	10	15	16
Atorvastatin	Amlodipine-d4	Positive	559.1	440.2	10	81	10	31	16
Candesartan	Telmisartan-d3	Positive	441.0	263.1	20	76	10	17	8
Doxazosin	Amlodipine-d4	Positive	452.0	344.1	10	81	10	41	12
Eplerenone	Amlodipine-d4	Positive	415.0	162.9	10	136	10	23	15
Furosemide	Telmisartan-D3	Negative	329.0	204.8	30	−30	−10	−32	−13
Hydrochlorothiazide	Telmisartan-d3	Negative	295.9	268.9	50	−130	−10	−28	−13
Chlortalidone	Amlodipine-d4	Positive	338.8	242.9	30	86	10	35	14
Indapamid	Indapamide-d3	Positive	366.1	131.9	10	61	10	21	8
Lercanidipine	Amlodipine-d4	Positive	612.2	280.2	10	66	10	33	8
Losartan	Telmisartan-d3	Negative	421.1	126.9	30	−95	−10	−38	−11
Losartan metabolite	Telmisartan-d3	Negative	435.1	157.0	30	−35	−10	−32	−13
Nitrendipin	Nitrendipine-d5	Positive	361.2	315.0	10	66	10	13	20
Perindopril	Perindopril-d4	Positive	369.2	172.1	10	61	10	29	12
Perindoprilate	Perindoprilat-d4	Positive	341.3	170.0	10	71	10	25	12
Ramipril	Ramipril-d5	Positive	417.2	234.1	10	66	10	29	16
Ramiprilate	Ramiprilat-d5	Positive	389.3	206.1	10	51	10	29	14
Rosuvastatin	Amlodipine-d4	Positive	482.0	258.0	30	76	10	45	8
Sacubitril	Perindopril-d4	Positive	412.1	266.0	10	86	10	40	8
Sacubitrilat	Perindoprilat-d4	Positive	384.0	266.1	10	111	10	40	10
Spironolactone	Spironolactone-d6	Positive	341.2	107.1	10	91	10	43	8
Telmisartan	Telmisartan-d3	Positive	515.2	497.3	5	70	10	47	6
Trandolapril	Perindopril-d4	Positive	431.1	234.1	10	91	10	29	8
Trandolaprilat	Perindoprilat-d4	Positive	403.1	170.1	20	50	10	27	18
Valsartan	Telmisartan-d3	Positive	436.1	291.1	5	86	10	25	10
Amlodipine-d4		Positive	413.1	237.8	20	46	10	15	16
Indapamide-d3		Positive	368.8	135.0	20	61	10	23	2
Nitrendipine-d5		Positive	366.1	315.0	10	61	10	13	22
Perindoprilat-d4		Positive	345.1	170.0	20	61	10	25	12
Perindopril-d4		Positive	373.2	176.1	20	56	10	29	12
Ramiprilat-d5		Positive	394.2	211.1	20	61	10	29	14
Ramipril-d5		Positive	422.2	239.0	20	66	10	31	16
Spironolactone-d6		Positive	347.1	107.0	30	91	10	43	8
Telmisartan-d3		Positive	518.2	500.4	5	70	10	47	6
Telmisartan-d3		Negative	516.2	288.9	30	−125	−10	−46	−23

Q1, precursor ion (m/z); Q3, fragment ion (m/z); DP, declustering potential (V); EP, entrance potential (V); CE, collision energy (V); CXP, cell exit potential (V).