Antidepressant Therapeutic Drug Monitoring by Minimally Invasive Techniques in Eating Disorders Patients: Preliminary Results from a Pilot Study with Focus on Vortioxetine

Fabio Panariello[1]*, Tomas Mastellari[2], Angela di Gianni[2], Maurizio Speciani[2], Michele Protti[3], Laura Mercolini[3], Diana De Ronchi[2] and Anna Rita Atti[2]

1Mental Health Department, Local Health Authority, Italy

2Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy

3Research Group of Pharmaco-Toxicological Analysis (PTA Lab), Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Italy

*Corresponding author: Fabio Panariello, Mental Health Department, Local Health Authority, Italy

ARTICLE INFO

ABSTRACT

Therapeutic Drug Monitoring (TDM) is an evidence-based practice consistent with the assumption that pharmacological plasmatic concentrations correlate better with clinical effects than prescribed doses of the used drugs. TDM has several indications in treated psychiatry patients: comorbidities, suspected non-compliance, severe adverse effects and tailored pharmacotherapy. Antidepressant Drugs (AD) are prescribed in patients with Eating Disorders (ED) to treat depression or anxiety disorders associated with ED or to reduce binge-eating behaviours. TDM may represent a valid tool in this population, considering the limited efficacy of ED's pharmacological treatment and the high rate of adverse effects. Nineteen outpatients affected by ED with a Body Mass Index (BMI) < 20 or > 30 kg/m^2 treated with antidepressants agreed to participate in this study. Participants were treated with Sertraline (N=5), Fluoxetine (N=6), Vortioxetine (N=4), Citalopram (N=2), Escitalopram (N=1), Fluvoxamine (N=1). Oral fluid samples and whole blood dried microsamples by finger puncture using VAMS (Volumetric Absorptive Microsampling) technique were obtained from patients. Sociodemographic and clinical information were also collected.

Preliminary results by our pilot study show a correlation between plasmatic and salivary concentrations only for Vortioxetine but not for other examined antidepressants. Additionally, plasmatic concentrations of all examined antidepressants are constant for extreme BMI when dose-corrected. By considering these preliminary data, we are confident that further studies characterized by the expansion of the sample size will allow us to outline that TDM may represent a valid tool in order to better explain the limited efficacy of AD in patients with starvation state or obesity.

Introduction

Patients with Eating Disorders (ED) including, among others, Anorexia Nervosa (AN), Bulimia Nervosa (BN) and Binge-Eating Disorder (BED) suffer from a persistent disturbance of eating-related behavior that results in the altered consumption or absorption of food and that significantly impairs physical health and/or psychosocial functioning [1]. The latest guidelines regarding ED [2] recommend a multidisciplinary intervention, including psychological treatment, dietary advice and medication. Despite the body of research on ED psychopharmacologic treatment has increased lately, there remains a paucity of appropriately sized Randomized Controlled Trials (RCTs) and a great need of further studies on pharmacotherapy agents in ED [3], and especially in

AN [4-6]. Even if many medications have been used in patients affected by AN, outcomes are generally poor. The lack of response to medications may be the result of the complicated physiological state of starvation [5].

Fluoxetine, for example, which is the only Selective Serotonin Reuptake Inhibitor (SSRI) approved to treat BN [7], does not increase patients' Body Mass Index (BMI) and does not show any significant psychopathological improvement in patients with AN [5]. Current treatment guidelines do not recommend the use of SSRIs as unique therapeutic approach for AN due to limited efficacy [2,8] and increased risk of adverse effects [9]: there is a lack of evidence for the use of SSRIs in underweight patients for specific AN-related symptoms [8], although the use of SSRIs may help in relapse prevention and improvement of psychiatric comorbidities (eg. depression, anxiety and OCD) [6,8], above all when weight is restored. Nevertheless, SSRIs are actually the most common category of psychotropic drugs prescribed in patients with ED [10,11], even during acute phases, explaining the so-called "research-practice gap" (patients not always receiving treatments rooted in scientific evidence) [12].

It was suggested that SSRIs have decreased efficacy in anorexic patients with low BMI due to starvation-related biochemical changes in the brain [13] and because of inadequate concentration of nutrients, which are necessary for serotonin metabolism [14]. Nevertheless, nutritional supplements containing tryptophan and essential fatty acids do not seem to increase SSRIs efficacy in underweight patients with AN [15]: the potential reason remains uncertain, but the latest genetic findings suggest that ED may not only be seen as psychiatric, but also as metabolic and immune disorders [6,16]. In a study on patients treated by Risperidone, Paulzen M. and colleagues suggested that extreme low and high BMIs modify psychotropic drugs' metabolism, in particular pharmacokinetic parameters [17]: obese patients showed a higher plasma concentrations of the active metabolite of Risperidone when compared with cachectic patients; authors speculate that this pharmacological behaviour is due to cytochrome P450 activity alterations or differences in P-glycoprotein function. Other studies showed negative correlation between BMI and plasma concentration of psychotropic drugs and/or their metabolites [18,19] and these results are explained by the authors as linked to different distribution volumes for lipophilic substances (larger distribution volume in obese subjects leading to inadequate plasma levels and smaller therapeutic efficacy) [19]. Unterecker et al. instead point out the absence of any relationship between body weight and plasma concentrations of antidepressants, highlighting the need for further exploration studies [20]. Vortioxetine is an antidepressant with multimodal activity currently approved for the treatment of major depressive disorder that is metabolized by cytochrome P450 enzymes and subsequently by uridine diphosphate glucuronosyltransferase. Several evidences from current literature do not show clinically relevant differences

in vortioxetine exposure by sex, age, race, body size, and renal or hepatic function. Dose adjustment is only recommended for cytochrome P450 2D6 poor metabolizers and when it is associated with bupropion, a strong cytochrome P450 2D6 inhibitor, and rifampin, a broad cytochrome P450 inducer.

The simultaneous administration of other drugs does not affect vortioxetine exposure or safety profile. Moreover, pharmacodynamic findings demonstrate that vortioxetine achieves high levels of serotonin transporter occupancy in relevant brain areas and modified abnormal resting state networks in the brain over the therapeutic dose range. Overall, vortioxetine can be administered without major dose adjustments [21]. Considering the altered physical and metabolic condition of patients with extremely low and high BMIs, we aimed to investigate the usefulness and feasibility of minimally-invasive and miniaturised biosampling techniques used for Therapeutic Drug Monitoring (TDM) of psychiatric Central Nervous System (CNS) drugs [22,23] in outpatients affected by ED. TDM of SSRIs has already several approved indications such as lack of clinical response despite adequate doses, adverse effects using recommended doses, and drugs' prescription in patients with pharmacokinetically relevant comorbidities (eg. extremely high or low BMI) [24,25], to obtain better therapy optimization and personalization [25,26]. Moreover, TDM can also lead to reduced healthcare expenses, due to the possibility of better efficacy, increased patient compliance and enhanced safety, leading to a reduction in hospitalizations due to unwanted effects or therapy ineffectiveness [27-30].

In this pilot study we have described the use of Volumetric Absorptive Microsampling (VAMS) in the management of pharmacological treatment of ED patients in outpatient setting in order to analyse any pharmacokinetic differences of the main antidepressants in use in patients with very varied ED and BMI. A secondary goal is to test for the first time the applicability of the VAMS technique for the analysis of antidepressants in whole blood and Oral Fluid (OF) for the purposes of TDM. The main aim could help us to better describe and understand the relationship between the BMI and biological fluid concentrations of antidepressant drugs together with their metabolites in these cohort of patient. In order to do that, we have carried out an analytical workflow based on VAMS of both whole blood and OF, followed by microextraction by packed sorbent (MEPS) [23] and Liquid Chromatographic (HPLC) analysis with spectrophotometric (UV) and spectrofluorimetric (FL) detection.

Experimental Procedures

Participant's Enrolment

From January 2019 to May 2019, a total of 19 patients consecutively evaluated at the outpatient Unit for Eating Disorders of Bologna University (Northern Italy) were enrolled in the present study. This outpatient unit for ED is an academic clinic specialized

in the diagnosis and treatment of ED in adult individuals (≥18 years old). Patients were asked to participate in the study whether they met the following inclusion criteria:

a) a diagnosis of Eating Disorder according to DSM-5 criteria(American Psychiatric Association, 2013),

b) BMI < 20 kg/m2 or > 30 kg/m^2,

c) age ≥ 18 years and ≤ 30 years,

d) pharmacological treatment (SSRIs or vortioxetine).

Exclusion criteria were the presence of other several mental disorders, in particular schizophrenia, substance abuse and bipolar disorder.

Patient's Assessment

The evaluation protocol at the outpatient unit for ED consisted of three different meetings with a psychiatrist. In the first meeting, socio-demographic and clinical data were collected from the patients and from their medical records; during the second consultation, we filled in a psychopathological assessment including some specific tools for ED; finally, during the third meeting the psychiatrist shared the diagnostic conclusions with the patient and suggested the type of treatment. In case of eligibility for the present study, a fourth meeting was scheduled for the biosampling session. During this last consultation, additional information were collected by the physician, including the type of antidepressant drug, dose, last intake, first prescription of the treatment and compliance.

Ethical Issue

The present study was approved by the local Ethical Committee (Comitato Etico Indipendente di Area Vasta Emilia Centro, code: CE18135) and all recruited patients signed the informed consent. All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975 and its most recent revision.

Biosampling

Patients participating in the present study were scheduled to be visited at the ED outpatient clinic in the morning between 10 a.m. and 12 a.m. Patients were informed neither to drink nor eat in the 30 minutes before the consultation. The intervention consisted in the microsampling of whole blood in dried form using VAMS approach and the collection of OF after reaching the steady-state for the antidepressant drug (fourth meeting). In fact, TDM procedures must be carried out under steady-state condition, which is reached within a time of 4-5 half-lives [22]. For different SSRIs and vortioxetine, at least 14 days of treatment are necessary to get to steady-state conditions.

Blood drops were obtained by minimally invasive finger pricking by means of a sterile, disposable needle, while whole blood biosampling was carried on using specific devices based on VAMS technology. VAMS devices are particularly easy to handle and facilitate the contact between the polymeric head of the device ("tip") and blood drops. In the present study, three 20-µL tips were obtained for each patient, representing identical sample replicates. Simultaneously, OF was collected by spitting in a dedicated microtube. VAMS devices were then stored in a tightly closed container and in the dark at room temperature. Storage clamshells provided as part as VAMS packages were used in the present study before the analysis process, which was carried out by the research group of Pharmaco-Toxicological Analysis (Department of Pharmacy and Biotechnology, FaBiT – University of Bologna). On the other hand, OF was stored at the temperature of 0°C before transportation to the Analysis Laboratory. Analysis were carried out by means of fully validated analytical methodologies.

Materials

Duloxetine hydrochloride (used as the internal standard IS1 for HPLC-UV) and Venlafaxine hydrochloride (used as IS for HPLC-FL), pure powders (all >99% purity); acetonitrile, methanol and dichloromethane (for HPLC, purity: > 99.9%), monobasic potassium phosphate, Triethylamine (TEA), phosphoric acid, sodium carbonate and potassium hydroxide (all pure for analysis) were purchased from Sigma Aldrich Italy (Milan, Italy). Clotiapine (used as IS2 for HPLC-UV) pure powders were purchased from LGC Standards (Teddington, Middlesex, UK). Ultrapure water (18.2 MΩ cm) was obtained by means of a Milli-Q apparatus from Millipore (Milford, MA, USA). ISS stock solutions (1 mg/mL) were prepared by dissolving suitable amounts of pure powders in methanol and kept at -20°C when not in use; the corresponding standard solutions were prepared daily by dilution with the HPLC mobile phase. All solutions were stored protected from light in amber glass vials from Phenomenex (Torrance, CA, USA).

Hplc-Uv- Fl Instrumentation And Conditions

HPLC-UV- FL analysis was performed on a Waters Corporation (Milford, MA, USA) Alliance e2695 chromatographic system with autosampler coupled to a Waters 2998 photo diode array detector and a Jasco FP-2020 spectrofluorometric detector, connected in series. Separations were obtained on a Waters SunFire C18 column (100 x 3.0 mm, 3.5 µm) maintained at room temperature and equipped with a guard column. The mobile phase was a mixture of 33 mM, pH 3.0 aqueous phosphate buffer containing 0.3% TEA (solvent A) and acetonitrile (solvent B), flowing at a constant rate of 1.0 mL/min under gradient conditions. Gradient composition was: 0.0-3.0 min, constant 20% A; 3.1-4.0 min, linear 20%-35% A gradient; 4.1-6.5 min, constant 35% A; 6.6-7.5 min, linear 35%-55% A gradient; 7.6-14.5 min, constant 55% A; 14.6-15.5 linear 55%-20% A gradient, 15.6-17.0 constant 20% A to re-equilibrate the column. Both solvents were filtered on a polyamide filter (47 mm dimeter, 0.2 µm) and degassed by ultrasonication. Injection

volume was 20 µL. Sertraline (SRT), Norsertraline (NSR) and Vortioxetine (VTX) were detected by UV at 225 nm; Fluoxetine (FLX), Citalopram (CTP), Norfluoxetine (NFL), Dextcitalopram DCT and Diethyldithiocarbamate (DDC) were detected by fluorescence at λem = 235 nm, λexc = 300 nm.

Blood- and OF-VAMS Sampling and Pretreatment

For patient sampling, IS spiking was carried out on the VAMS tip by automatic pipetting before the sampling; the tip was then left to dry for 2 h at RT before use. Mitra® VAMS microsamplers (20 µL) were provided by Neoteryx (Torrance, CA, USA). A VAMS microsampler includes a polypropylene handle (about 4 cm long) topped with a small tip (about 2-mm diameter) of a proprietary polymeric porous material. B-VAMS. The finger prick site was wiped with skin cleansing swabs and dried. Then a disposable sterile lancet was used to prick the fingertip and a blood droplet was allowed to form. Each VAMS tip was held at a 45° angle to the surface of the blood droplet, taking care not to touch the skin. The VAMS devices were held in this position until the whole tips were visible filled with blood (around 5 seconds). The samplers were then transferred to the dedicated clamshells, in order to avoid contact with any surface and left to dry at Room Temperature (RT) for 1 hour.

VAMS microsamples were thus obtained. Clamshells were stored and transported at RT in sealed polyethylene bags containing desiccant until pretreatment and analysis. For sample pretreatment, the microsampler tip was detached from the handle and subjected Ultrasound-Assisted Extraction (UAE) for 20 min in 1 mL of methanol. The resulting solution was quantitatively transferred into a different vial and brought to dryness in a centrifugal evaporator. After re-dissolving with 100 µL of HPLC mobile phase, the solution was subjected to Microextraction by Packed Sorbent (MEPS) pretreatment in an SGE Analytical Science (Melbourne, VIC, Australia) C2 barrel-and-needle (BIN) assembly set up in an SGE eVol XR digital analytical syringe apparatus. The BIN was activated by drawing and discharging 100 µL of methanol 3 times and conditioned with 100 µL of water 3 times. The sample was loaded onto the BIN with 10 draw/discharge cycles at a 5 µL/s speed; the BIN was then washed twice with 100 µL of water and 100 µL of 10 mM, pH 9.0 carbonate buffer / methanol (90/10, V/V)

mixture at 20 µL/s. The analyte and the ISs were eluted three times with 200 µL of methanol at 5 µL/s (three cycles). After merging the three eluates, they were brought to dryness, re-dissolved in 100 µL of HPLC mobile phase and analysed by HPLC-UV-FL. OF-VAMS. OF (1 mL) aliquots were centrifuged for 5 min at 6500 x g, then VAMS samplers were used by touching the OF sample surface with the tip and held in position for 10 seconds. Then, the same drying, storage, pretreatment and analysis workflow as B-VAMS was carried out.

Statistical Analysis

All the statistical analysis were performed using SPSS IBM V.24.0 (statistical Package for Social Science). Other analysis (i.e. Linear Regression) will be performed in further studies when the sample size will be compatible with statistical power.

Results

As reported in Table 1, 84.21% of enrolled patients are female. 47% of patients are affected by Anorexia Nervosa (AN), while 15% were diagnosed with bulimia nervosa and 38% with Binge Eating Disorder (BED). Median age of patients enrolled is 26 years, with a minimum age of 19 and a maximum of 52 years. Median weight is instead of 48 kg, with a minimum value of 38 kg and a maximum of 140 kg. Median BMI is 18.4 kg/m² (underweight), with a minimum BMI of 14.1 kg/m² (severe thinness) in a patient with AN and maximum BMI of 47.3 kg/m² (severe obesity) in a patient with BED. As reported in Table 2, in order to treat psychiatric comorbidities or specific psychopathological issues of ED, we have treated our patients with one or more (1 patient) antidepressant drugs. 26.32% of patients is treated with vortioxetine (to treat major depression comorbidity in two patients with AN, one patient with BN and two patients with BED); 31.58% of patients take fluoxetine (in 2 patients with AN to treat depressive symptoms, in 1 patient with BN and 3 patients with BED to reduce binge eating behaviour); 26.32% of patients is treated with sertraline (to treat anxiety-depressive symptoms in 3 patients with AN, 1 patient with BN and 1 patient with BED); the 10.53% of patients is treated with Citalopram (in two patients with AN to treat anxiety symptoms); finally, one patient is treated with Escitalopram and one patient with Fluvoxamine (to treat the concomitant obsessive-compulsive disorder in comorbidity with BED).

Table 1: Socio-demographic and clinical data.

Sex	Age	ED	W (kg)	H (cm)	BMI (kg/ m²)	Cigarettes per day	Other drugs
F	19	AN	38	164	14.1	0	Paracetamol, Nutritional supplements
F	45	AN	43	169	15.1	0	Trazodone
F	20	AN	38	153	16.2	0	Estroprogestinics
F	23	AN	41.5	160	16.2	0	
F	45	AN	47.5	167	17	0	B, D vitamins, Natural laxative
F	33	BN	43.4	159	17.2	20	
F	20	AN	48	166	17.4	7	Sulfasalazine, Trimebutine

| F	19	BN	54	175	17.6	10	
F	26	AN	47	163	17.7	2	
F	43	AN	47	160	18.4	0	Levothyroxine
F	23	BN	47	154	19.8	0.7	Delorazepam
F	22	AN	57	169	19.9	5	B9, B12 vitamines
M	18	BED	95	175	31	0	Risperidone
F	40	BED	92	165	33.8	20	Estroprogestinics
F	22	BED	95	161	36.6	0	
F	42	BED	103	164	38.3	0	Nimesulide
F	32	BED	129	175	42,1	0	Aripiprazole, Sodium valproate, Levothyroxine, Losartan, Bisoprolol, Estroprogestinics
M	52	BED	125	163	47	0	Insuline, Metformine, Statines, Ezetimibe, Levothyroxine, Furosemide, Ramipril, Cardioaspirine, Tiotropium, Salmeterole-Fluticasone, Zolpidem
M	32	BED	140	172	47,3	0	Allopurinol

Table 2: AD's prescription.

AD	Since weeks (N)	Daily dosage (mg)	Dosage since weeks (N)	Δt (h)
Vortioxetine	4	3.84	2	6.5
Fluoxetine	44	10	4	6.5
Sertraline	44	100	8	4.5
Sertraline	>48	100	20	3
Fluoxetine	40	60	36	2
Sertraline	4	50	2	4
Sertraline	2	50	2	22
Fluoxetine	>48	20	4	11
Citalopram	12	20	8	17
Citalopram	>48	10	6	9.5
Vortioxetine	2	7.68	2	0.5
Vortioxetine	12	15	4	1.5
Fluvoxamine	16	100	12	17
Fluoxetine	32	40	28	2
Fluoxetine	34	40	2	8
Escitalopram	40	10	12	3
Sertraline	28	100	24	7
Fluoxetine	6	20	6	2.5
Vortioxetine	>48	3.08	>48	5

Biological sampling was performed in all patients after reaching steady state. The average time calculated between the start of treatment (Time 0) and the biological sampling is 1.5 months, with a minimum time of 2 weeks for sertraline, vortioxetine and fluoxetine and a maximum time of 24 months for vortioxetine. This duration could correlate with the amount of the drugs and their metabolites that may have stored in the body, on the basis of their pharmacokinetic characteristics. As shown in Table 2, Vortioxetine has been prescribed in our population at a median dosage of 7.5 mg/day, while the median of the sertraline dosage is 100 mg/day. Fluoxetine is prescribed at a median dosage of 30 mg/day. Citalopram and escitalopram were both prescribed at a daily dose of 10 mg, Fluvoxamine at 100 mg. The time, expressed in hours, between the last intake of the drug and the collection of the bio-samples was variable (median of 5 hours, minimum value of 30 minutes and maximum of 22 hours).

In our sample, 36.8% of patients are smokers, while no patient shows alcohol abuse. The information regarding the additional drugs was taken, noted with the dosage and time of intake, and sent to the analysis laboratory, so that any drug interactions potentially altering the results of blood and OF concentrations can be taken into account. In Table 1, additional drugs taken by patients are shown as an indication and simplified. 47% of enrolled patients take other drugs daily. This high rate recall the importance and the

frequency of psychiatric and/or medical comorbidities in the cohort of patients affected by ED and reinforce the rationale of this study, i.e. the use of TDM in this specific population (Table 3). The most informative results were obtained from four patients treated with Vortioxetine. The first one is a 19 years old patient, female, suffering by AN with BMI of 14.1 kg/m². She has been treated with Vortioxetine 5 mg/die for a month. The second one is a 22-year-old female patient diagnosed with AN (BMI=19.9 kg/m²), treated with 15 mg/die of Vortioxetine. The third patient is a 23-year-old female subject affected by BN (BMI=19.8 kg/m²), treated with Vortioxetine at a dosage of 15 mg/die. Finally, a 32-year-old male patient affected by BED (BMI=47.3 kg/m²) has been taking 4 mg/day of vortioxetine for a long time. The collection of bio-samples took place between 30 minutes and 6.5 hours after the last intake of the drug, considering that vortioxetine reaches the maximum whole blood concentration between 7 and 11 hours after the intake. The ratio between the whole blood and salivary concentrations of Vortioxetine in the examined patients is close to the unit as showed in Figure 1. Further studies will allow us to expand the sample in order to quantify this trend and to proceed with statistical analysis with the aim of determining the statistical significance, supporting the potential use of OF despite to the blood sample for TDM of Vortioxetine.
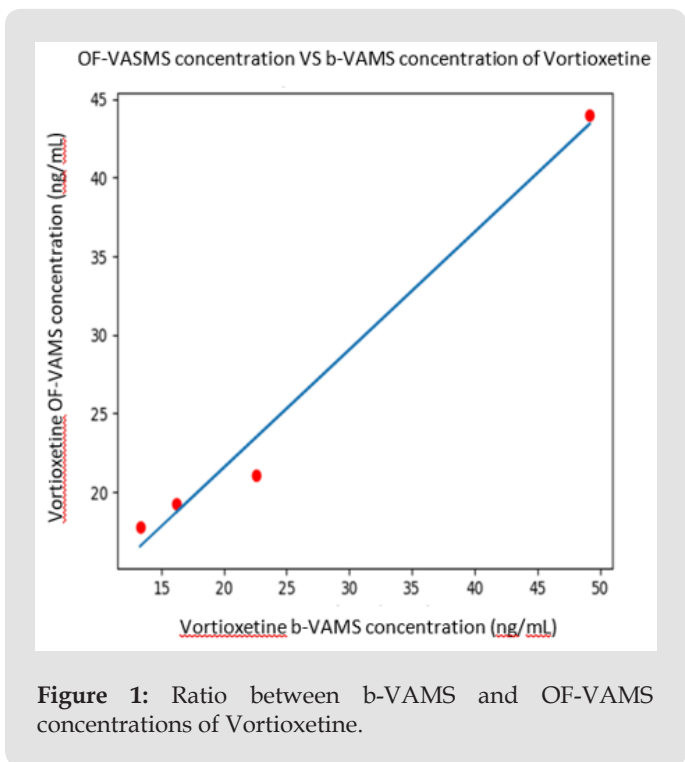
Figure 1: Ratio between b-VAMS and OF-VAMS concentrations of Vortioxetine.

Table 3: ordered by type of prescribed antidepressant and increasing BMI, shows the concentrations obtained from b-VAMS and OF-VAMS analysis, highlighting levels of the drug and any active metabolites.

Sex	Age	ED	BMI	AD	Dosage (mg)	Δt (hours)	AD ± metabolites (ng/ml) b-VAMS concentrations	AD ± metabolites (ng/ml) OF-VAMS concentrations
F	20	AN	16.2	Sertraline	100	4.5	120.0 ± Norsertraline: 119.3	8.8 ± Norsertraline: 10.4
F	23	AN	16.2	Sertraline	100	3.25	119.8 ± Norsertraline: 113.4	17.6 ± Norsertraline: 7.6
F	33	BN	17.2	Sertraline	50	4.25	59.2 ± Norsertraline: 14.5	26.7 ± Norsertraline: 28.5
F	20	AN	17.4	Sertraline	50	22	41.3 ± Norsertraline: 43.2	48.9 ± Norsertraline: 47.3
F	32	BED	42.1	Sertraline	100	7.25	111.9 ± Norsertraline: 117.0	22.5 ± Norsertraline: 14.5
F	45	AN	15.1	Fluoxetine	10	6.75	49.8 ± Norfluoxetine: 49.3	43.7 ± Norfluoxetine: 38.7
F	45	AN	17	Fluoxetine	60	2	75.0 ± Norfluoxetine: 61.8	16.6 ± Norfluoxetine: 13.2
F	19	BN	17.6	Fluoxetine	20	11	11.7 ± Norfluoxetine: 12.5	8.7 ± Norfluoxetine: 8.1
F	40	BED	33.8	Fluoxetine	40	2	73.2 ± Norfluoxetine: 50.2	33.0 ± Norfluoxetine: 20.7
F	22	BED	36.6	Fluoxetine	4	8	15.4 ± Norfluoxetine: 14.0	19.3 ± Norfluoxetine: 11.8
M	52	BED	47	Fluoxetine	20	2.5	58.5 ± Norfluoxetine: 13.7	36.5 ± Norfluoxetine:3.9
F	19	AN	14.1	Vortioxetine	5	6.5	16.2	19.3
F	23	BN	19.8	Vortioxetine	10	0.5	22.6	21.1
F	22	AN	19.9	Vortioxetine	15	1.5	49.2	44.0
M	32	BED	47.3	Vortioxetine	4	5	13.3	17.8
F	26	AN	17.7	Citalopram	20	17	45.5 ± Desmethylcitalopram: 15.6 ± Di-Desmethylcitalopram: 14.9	22.5 ± Desmethylcitalopram: 4.6 ± Di-Desmethylcitalopram: 2.2
F	43	AN	18.4	Citalopram	10	9.75	32.1 ± Desmethylcitalopram: 14.1 ± Di-Desmethylcitalopram: 11.3	33.1 ± Desmethylcitalopram: 3.1 ± Di-Desmethylcitalopram: 2.8

| F | 42 | BED | 38.3 | Escitalopram | 10 | 3 | 25.5 ± Desmethylcitalopram: 10.1 ± Di-Desmethylcitalopram: 3.7 | 20.6 ± Desmethylcitalopram: 3.8 ± Di-Desmethylcitalopram: 2.6 |
| M | 18 | BED | 31 | Fluvoxamine | 100 | 17 | 110.0 | 33.7 |

Considering a linear kinetic of Vortioxetine if we compare the corrected whole blood concentration of the drug for a dose of 5 mg/day to BMI, it emerges a higher ratio between whole blood Vortioxetine values and BMI in the patient with lower BMI (14.1 kg/m^2) than that with the highest BMI (47.3 kg/m^2). The ratios are 1.15 and 0.35. Therefore, considering the very similar dose prescribed to the two patients with extreme BMI, we obtain in the underweight patient a whole blood concentration of Vortioxetine corrected for a dose comparable to that of the obese patient (16.2 ng / ml against 16.62 ng/ml respectively). Furthermore, in underweight patients, the free component of the drug may increase due to the reduced amount of albumin and circulating proteins available to bind the drug. The mean values of maximum whole blood concentration of vortioxetine obtained after multiple administrations of 5, 10 or 20 mg/day are typically between 9 and 33 ng/ml, according to the literature (Banca Dati Farmaci dell'AIFA). Taking into consideration that the interval between the intake of the drug and the evaluation of the whole blood concentration could differ significantly, we note that the patient with AN (BMI 19.9 kg/m^2) has whole blood drug levels higher than the standard therapeutic interval (49.2 ng / ml), in the specific case the collection had taken place 5 hours after the last intake of the drug when the blood concentration has not yet reached the maximum level (Tmax between 7 and 11 hours after taking). The data of the high blood concentration of Vortioxetine per oral dose in the dosage range appears significant due to fact that it does not appear to be attributable to pharmacodynamic or pharmacokinetic interactions of the molecule (the patient does not take other drugs or substances).

Discussion

The main goal of this study is to highlight a possible relationship between the whole blood and salivary concentration of antidepressants and their active metabolites using minimally invasive TDM methods such as VAMS strategy in a specific population affected by ED. This subset of psychiatric patients is extremely interesting in terms of correlation of peripheral concentration of drugs with oral dosage, because patients with ED have an important variability in terms of BMI. It is well known that BMI could affect clinical response to antidepressant drugs [31,32]. TDM can give us correct information on the concentration of active metabolites of antidepressants in peripheral matrices, net of the effect of liver metabolism and BMI on the pharmacokinetics of psychotropic drugs.

VAMS technology is currently a topic of enormous interest for the bioanalytical community and is the subject of numerous studies that have grown exponentially in the last two years [23,33-36]. VAMS devices were initially marketed for use on whole blood only; this method based on VAMS devices is applied also to the OF for the quantitative analysis of synthetic cathinones [36]. The aim of the present study, evaluating the concentration of antidepressants through VAMS devices both on whole blood and on OF, is to:

a) examine the relationship between the concentration of drugs (above all Vortioxetine) and any active metabolites in the two biological matrices and oral dosages of the same drugs;

b) analyze any pharmacokinetic differences of the main antidepressants in use in patients with very varied ED and BMI.

Current findings in the literature concerning the correlation between whole blood and OF concentrations of psychotropic drugs outline information characterized by some limits. Often the data are fragmented, take into account very limited populations, or do not take into account the active metabolites of the drugs: some positive correlations have been highlighted between the whole blood and salivary concentration of monohydroxycarbamazepine [37], carbamazepine, phenytoin and phenobarbital [6,38]. For amitriptyline, nortriptyline and valproic acid, however, the correlation shown was not significant [6,38,39]. For a long time the concentration of drugs in OF has been considered as a reflection of the free component of the drug in the blood, which for many psychotropic drugs is only 10% or less of the total concentration; however, the distribution of drugs between blood and OF largely depends on the pH, which increases when the secretion is stimulated. OF is considered as an interesting and advantageous matrix for TDM studies, in particular due to the non-invasiveness, but data available so far in literature show contrasting results and further studies are necessary to validate the use of OF as a matrix for the TDM of antidepressant drugs [6,40]. On the other hand, according to the current literature [32], high BMI may also affect treatment response: Khan et al., [32] have found obesity to be associated with a less vigorous treatment response, with an effect more pronounced in males. Moreover, higher BMI was associated with a slower clinical response in the Munich Antidepressant Response Study (MARS) [41]. Finally, in the Genome Based Therapeutic Drugs for Depression (GENDEP) study, obesity was associated with a poorer response to nortriptyline in men and women, and a poorer response to escitalopram in women [19].

Several Proof-Of-Concept studies can be found in literature, demonstrating the usefulness of VAMS devices for various compounds and purposes, including the analysis of caffeine, paraxantine, steroid compounds, 5-methyltetrahydrofolic acid,

hydroxyurea, natural and synthetic cannabinoids, cocaine and metabolites, oxycodone and metabolites, asenapine enantiomers and iron isotopes [35,42-48]. To date, there are no studies on reliability of VAMS technique for the analysis of antidepressant concentrations, neither on blood nor on OF. In this context, this study may be considered as a true innovative pilot study for the usefulness and reliability of VAMS strategy within the TDM of psychotropic drugs, above all in patients with a wide range of BMI and pharmacokinetic characteristics.

In this context, our pilot study provides data about application of VAMS for OF analysis, showing a good relationship between whole blood and salivary concentrations for Vortioxetine only. The ability to outline the usefulness of VAMS on OF may have been hindered by the relatively small sample size. Future work should seek to enrich the patient sample and to standardise the time of data collecting and the last drug intake. The second macro-objective of the study concerns the evaluation of the pharmacokinetic behavior of antidepressant drugs in patients with extreme BMI (less than 20 kg/m^2 and greater than 30 kg/m^2). As explained in the introduction, the role of body weight and BMI on the distribution and therefore on the effectiveness of psychotropic drugs is particularly ambiguous and relatively little debated in the literature; the results of the few published studies are often incomplete and/or conflicting. The present TDM study of antidepressant drugs in patients with ED and therefore abnormal BMI allows us to:

a) Test in clinical practice the use of TDM in the pharmacological treatment of patients with abnormal body weight such as ED patients;

b) Contribute to the complex analysis of the factors that modulate the clinical efficacy of psychotropic drugs through a mainly pharmacokinetic perspective in a patient population particularly resistant to drug treatment.

In relation to point 1., we can state that TDM is characterized as a potential Precision Medicine tool, capable of reducing the Trial-and-Error approach and of favoring the therapist's choices in an Evidence-Based prospective above all in outpatient setting where the patients have a low body weight and show ED symptomatology with several psychopathological dimension symptoms. According to the guidelines, and even more so with respect to such fragile patients, often with physical comorbidities and with very low BMI, the psychiatrist's habit is to prescribe minimal doses of antidepressant drug (SSRIs, vortioxetine, etc.) and to begin a progressive and slow titration of the dosages. Often, the patient is not affected by any benefit or only reports the appearance of undesirable effects. When the medium-low dose drug treatment is not effective, the therapist must empirically evaluate whether to increase the dosage or to change the type of psychiatric drug. The clinician's resistance in prescribing the highest dosages allowed for a given antidepressant to patients with very reduced BMI recalls

the general principles of pharmacology according to which low distribution volumes (AN patients) will correspond to higher blood concentrations for a given dose of drug in ratio to patients with BMI in the normal range, population in which blood concentrations are studied with reference to a given oral dose.

In outpatient setting, a fortuitous trial-and-error process is used to try to reach the optimal dose for the specific patient [26,49]. This process does not favor the therapeutic alliance and explains the difficulties of treatment and healing. Instead, the psychiatrist can make targeted decisions relying on non-invasive and timely TDM methods: firstly, to rule out that the patient is not taking the drug at all or that he is taking it incorrectly (as discussed in the introduction part, adherence to pharmacological prescriptions are often scarce in this type of patients particularly ambivalent towards the process of change towards healing); secondly, to increase the dosage when whole blood concentrations of the antidepressant are too low or to change the type of drug if whole blood concentrations are approaching the upper limit of the therapeutic window, without showing the clinical expected results. In patients with high BMI we can also evaluate the whole blood concentration of the drug and therefore modify the daily dose or the molecule according to the pharmacokinetic characteristics of the individual patient.

In consideration of point 2., however, it is necessary to consider the pharmacokinetic variables and the absorption, distribution and elimination characteristics of the antidepressant drugs considered in this study, described in the introduction, except for vortioxetine, chosen in this study, for the peculiar pharmacokinetic profile, as well as for the uniqueness of the pharmacodynamic profile [21]. Overall, the pharmacokinetic factors to be taken into account when describing the concentrations of antidepressants in blood and OF of such patients with particular physical characteristics are numerous and intricate. Ideally, the chemical-physical properties of the drug, its absorption, first pass metabolism, bioavailability, protein binding, distribution in abnormal body volumes (in association with the study of body composition), should be taken into account. The fat content and visceral obesity could explain some differences in the response to antidepressant drugs [19], the passage at the level of the blood-brain barrier to reach the target sites where the action is performed from a pharmacodynamic point of view, as well as the mechanisms of final metabolism and excretion. Added to this is the role of pharmacogenetics and the inter-individual variability, characterized by the different levels of cytochrome activity. Among other things, this activity is modified by obesity and the type of diet, as shown by various studies [50-52].

Our results, in line with recent literature data [20], support the absence of a significant influence of BMI on drug whole blood concentrations in relation to the oral posology taken. This data partially contrasts with the pharmacokinetic behavior generally expected by the clinician on the basis of the fundamental principles of pharmacology: in a large distribution volume (patient with BED)

the whole blood concentration of the drug will be lower than in a patient with a very poor volume distribution (patient with AN). Considering that routine blood tests are systematically prescribed during the taking in charge at the Unit for the Study and Assistance of the DCA and that patients are followed in an outpatient setting without the needing of hospitalization, it is possible to affirm with relative safety that the patients enrolled in the present study do not show significant alterations in renal and/or hepatic function, although the latter have not been thoroughly evaluated with specialized tests [53-55].

This pilot study reports numerous limitations for logistical reasons, having been conducted in a "real world" setting with all the difficulties associated with the organization of the collection of samples in an outpatient setting, the variability and inconstancy in taking the drug by patients, the timing of the withdrawal, difficult to agree with patients due to their unavailability. The limits also include devices and analysis procedures cost, and the low number and heterogeneity of the sample under study: the great variability of the BMI included, of the drugs taken and the medical comorbidities limit the possibility of examining correctly the relationship between drug concentrations in the matrices examined in such a small sample of patients. Our aim is to perform further studies in order to obtain a enriched sample size, a better standardization to reduce Δt variations among the sample of patients, the number and the differentiation of the various antidepressants in favor of an expansion of the cohort of patients who take Vortioxetine due to demonstrated tendency to provide a relationship that approximates the unity between salivary and whole blood concentrations.

Furthermore, the present study lacks correlations between clinical outcome and plasmatic antidepressant concentrations: in further studies it would be useful to consider using more specific psychiatric scales to accurately correlate efficacy and adverse effects to plasmatic antidepressant ranges. The applicability of effectiveness and minimally invasive TDM techniques opens the doors to the use of these tools in populations that are even larger and not spared by the appearance of ED, such as the pediatric one. The evaluation of whole blood and salivary concentrations of antidepressants in underweight or obese patients with ED allows us to formulate hypotheses and pharmacokinetic conjectures on the particular ineffectiveness of these drugs in ED patients. In this context, the present study and the thesis that derives from it intend to focus attention also on the need for further and more in-depth studies, which will explore the pharmacokinetic and pharmacodynamic mechanisms, as well as the neurobiological reasons underlying the strong resistance of the Disorders of the Food behavior to drug treatment. More studies in this direction are necessary to improve the global and multidisciplinary management of these disorders, characterized by the highest mortality rate in the field of psychiatric diseases and which today represent a real mental health emergency, as well as a problem of public health(EpiCentro - Istituto Superiore di Sanità).

Contributors

Panariello F. Analized the data, managed the literature searches and analyses and wrote the first

draft of the manuscript

Mastellari T., Wrote the protocol and collected the data

di Gianni A. Wrote the protocol and collected the data

Speciani M. Managed the literature searches

Protti M. Processed, analysed the samples and undertook the statistical analysis

Mercolini L. Processed, analysed the samples and undertook the statistical analysis

De Ronchi D. Designed the study and wrote the first draft of the manuscript

Atti AR Designed the study, wrote the first draft of the manuscript and undertook the statistical

analysis

All authors contributed to and have approved the final manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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