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## Contents

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REVIEW

# Cardiac Autonomic Neuropathy in Diabetes Mellitus Patients – Are We Aware of the Consequences?

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Cardiovascular autonomic neuropathy is the most frequent clinical form of autonomous diabetic neuropathy and appears secondary to cardiac autonomous fibre involvement, actively involved in cardiac rhythm impairment. Type 2 diabetes mellitus patients can present cardiac autonomic neuropathy early in the disease. Autonomous nerve function in DM patients should be assessed as early as the diagnosis is set in order to establish the optimal therapeutic strategy. The most frequent cardio-vagal test used is heart rate variability. An abnormal heart rate variability in the presence of orthostatic arterial hypotension indicates a severe cardiac autonomic neuropathy diagnosis. The development of cardiac autonomic neuropathy is subjected to glycaemic control, duration of the disease and associated risk factors. The glycaemic control is extremely important, especially early in the disease. Therefore, a poor glycaemic control carries unfavourable long-term effects, despite an ulterior optimal control, a phenomenon named "hyperglycaemic memory". In type 2 diabetes mellitus patients, the association of cardiac autonomic neuropathy with intensive glycaemic control increases the mortality rate, due to the fact, that, secondary to autonomous impairment, the patients do not present the typical symptoms associated with hypoglycaemia. Stratifying the cardiac autonomic neuropathy aids the clinician in assessing the morbidity and mortality risk of diabetes mellitus patients, because it is an independent risk factor for mortality, associated with silent myocardial infarctions and the risk of sudden death.

**Keywords**: cardiac autonomic neuropathy, diabetes mellitus, hyperglycaemia, hyperglycaemic memory, diabetic neuropathy

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## Introduction

Autonomous diabetic neuropathy is the least recognised and understood type of diabetic neuropathy (DN), despite a significant negative impact on long-term survival and quality of life of these patients. The autonomous nervous system virtually regulates the functions of all the organs, therefore, the autonomous diabetic neuropathy can lead to mild or dire consequences [1].

Cardiovascular autonomic diabetic neuropathy (CAN) is the most frequent clinical form of autonomous diabetic neuropathy, defined as an impairment of the autonomous control upon the cardiovascular system in diabetes mellitus patients (DM), after the exclusion of other systemic causes. The autonomous nervous system closely integrates vital processes such as cardiac rhythm, blood pressure (BP), myocardial contractility and in consequence, plays a primordial role in cardiovascular regulation. CAN, probably one of the most severe and neglected complications, appears secondary to cardiac autonomic fibre involvement, leading to an impairment of the cardiac rhythm and of the vascular dynamic [2,3,4,5].

The clinical manifestations of CAN have a significant impact upon the quality of life of these patients and are associated with a poor outcome, due to orthostatic arterial hypotension, intolerance to physical activity, silent myocardial infarctions, malignant cardiac arrhythmias and sudden death. Usually, the symptoms of CAN appear in the late stages of DM, but a subclinical CAN can be detected in DM patients one year after the initial diagnosis. This aspect underlines the importance of CAN screening [1,6,7]. Once the diagnosis of CAN has been established, the patients need to be closely monitored for physical activity, early identification of silent myocardial ischemia, assessment of the current medication and careful control of the associated cardiovascular risk factors [7,8].

Globally, the diagnosis of DM is an extremely important public health issue. The past decades recognised an increasing and dramatic change in this aspect, due to a continuous increase in the prevalence. This is most likely secondary to population ageing and a decrease in physical activity. Furthermore, the diagnosis of DM started to appear in younger individuals (40-64 years old), which leads to a longer duration of the disease and a higher risk of mortality and morbidity secondary to DM complications, especially due to cardiovascular diseases [9].

According to FID data, in Romania, the prevalence of DM for 2011 was 9.2% and is estimated that by the end of 2030, the prevalence will reach 11.1%. Globally, for each individual that receives the DM diagnosis, another one remains undiagnosed. Romania is in the top 10 in Europe for undiagnosed DM patients [10,11].

Considering these disquieting epidemiological data, together with the severity of CAN, this diagnosis needs to be prioritised by the clinicians involved: neurologists, diabetologists and cardiologists. The efforts must be joined together in order to extend the area of expertise and to

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positively influence the clinical evolution, for a better long-term prognosis of DM patients.

## CAN diagnosis

Symptoms and clinical signs of autonomic cardiac involvement

The symptoms of cardiovagal involvement can be tachycardia, dizziness, visual impairment and positional fainting when passing from a supine to a standing position [12]. A decrease in the heart rate variability (HRV) can be one of the first symptoms in CAN, reflecting the vagal dysfunction. In healthy subjects, an increase of the sympathetic activity will determine a reactive increase in the parasympathetic activity. In consequence, any increase in the sympathetic activity will lead to a reduced acceleration of the heart rhythm if the autonomic functions are intact. The rest tachycardia, with an occasional increase of the heart beats up to 130 beats per minute is characteristic for the late stages and is secondary to an increase in the sympathetic tonus associated with a vagal dysfunction. Clinically speaking, tachycardia in DM patients has to be considered a sign of autonomic dysfunction, even if any other potential cause has been ruled out (hyperthyroidism, anaemia, infections, cardiac insufficiency) [13,14,15]. "Fixed" tachycardia, which doesn't modify as a response to moderate physical activity, stress or sleep, indicates an almost complete cardiac denervation and has to be considered a sign of severe autonomic cardiovascular impairment [6].

Additionally, besides "fixed" tachycardia, the lack of an increase in the cardiac rhythm from passing from a supine to a standing position has significant clinical repercussions, due to the fact it involves a compensatory increase in the cardiac ejection, aggravating the orthostatic arterial hypotension, which usually accompanies the resting tachycardia [16,17]. Also, it has been demonstrated that the resting tachycardia is involved in the genesis of ventricular arrhythmias, with the consequence of sudden death [18].

### Cardiovascular reflex tests (Ewing tests)

The clinical research of the cardiovascular autonomic function significantly progressed based on the studies of Ewing and Clarke [19], who introduced a battery of cardiovascular reflex tests that have been successfully reproduced in a large number of studies. By using these tests in current clinical practice, the subclinical forms of CAN can be early detected, by identifying a reduction in the HRV [20, 21, 22].

Cardiovascular reflex tests record the HRV, notably the variability of the R-R interval during the Valsalva manoeuvre. This is recorded during deep breathing or during the change in posture, from a supine to a standing position, together with BP variability from a supine to a standing position during a sustained contraction of the dynamometer. The HRV predominantly (but not exclusively) records the parasympathetic nervous system function, and BP variability records the sympathetic nervous system function [5, 23]. Pafili et al, (2015) reported that deep breathing HRV is the best indicator for CAN, and that the accuracy of the diagnosis is increased if this technique is combined with HRV by deep breathing Valsalva manoeuvre [24].

### Spectral analysis of HRV

The spectral analysis of HRV represents a more advanced method of CAN diagnosis. This can be evaluated by breaking down the R-R interval and estimating the magnitude of the variability according to frequency. There are two main wavelength frequencies: (1) low-frequency (0.04-0.15 Hz) – vasomotor activity; (2) high-frequency (0.15-0.4 Hz) – synchronized with the breathing. The sympathetic system modulates the low-frequency HRV and the parasympathetic nervous system modulates the high-frequency. The magnitude of the spectral variation reflects the amplitude of cardiac rhythm fluctuation in different frequencies, during the breathing-in and breathing-out manoeuvre. This technique has the advantage that it doesn't require active participation from the patient, being evaluated in resting conditions [6, 25].

### Arterial baroreflex sensitivity assessment

The arterial baroreceptor reflex is responsible for maintaining short-term BP. By stimulating the baroreceptors, two major effector mechanisms are activated. The cardiac vagal fibre stimulation decreases the cardiac rhythm and in consequence, it increases the cardiac flow, while the inhibition of the sympathetic vasoconstrictor activity reduces the peripheral vascular resistance. The vagal tonus is maintained by various reflex mechanisms which are continuously activated by the stimulation of the baroreceptors. Therefore, the level of the vagal tonus is proportional to the sensitivity of the baroreceptors. The vagus nerve carries cardioprotective effects in order to maintain the electric stability of the myocardial muscle. An impairment in the baroreceptor sensitivity represents, therefore, a cardiovascular risk factor. In order to assess the sensitivity of the baroreceptors, the spectral analysis of the length of the R-R interval induced by spontaneous BP fluctuations is used. A specialised software analyses the sequences in which both the BP and the R-R interval simultaneously increase or decrease during three cardiac cycles [26, 27, 28].

### Measuring the corrected QT interval

The length of the QT interval is widely influenced by the autonomous nervous system tonus and a prolonged QT interval increases the risk of both severe cardiac arrhythmias and sudden death. A corrected QT over 440 milliseconds is considered abnormal. Evaluating the corrected QT interval can be a simple and useful instrument that can identify the patients that carry a high cardiovascular risk [29]. For the past years, the dispersion of the QT interval has been used – it analyses the difference between the longest and the shortest QT interval measured in a 12 lead EKG [30].

### Cardiac scintigraphy

Cardiac scintigraphy allows a complex imagistic assessment of the cardiac sympathetic innervation, by using sympathetic neurotransmitters analogues, such as I-metaiodobenzylguanidine (MIBG). A reduced or abnormal MIBG capture suggests an early sign of myocardial adrenergic innervation impairment. A good metabolic control improves these anomalies [27, 31, 32]. This method is far more sensible than the Ewing's battery and spectral analysis for the early CAN detection, but it requires advanced machinery and cannot be performed at a large scale.

## Stages of CAN and screening diagnostic tests

The presence of one abnormal cardiovagal test, such as HRV, indicates early, subclinical or probable CAN, which needs to be confirmed in time. In order to establish the diagnosis of definite CAN at least two serially cardiovagal tests need to be abnormal. The presence of orthostatic arterial hypotension (symptomatic or asymptomatic) indicates advanced or severe CAN diagnosis [20, 22].

The diagnostic protocol for CAN needs to be carried out in the following instances: (1) DM type 2 patients at the moment of diagnosis; (2) DM type 1 diagnosis at 5 years after diagnosis; (3) independent on the DM duration if there are signs of autonomic dysfunction; (4) diabetic patients that need to follow a restricted programme of physical exercises with moderate-high intensity, especially in the presence of associated cardiovascular risk factors; (5) DM patients with a history of impaired metabolic control, high cardiovascular risk and microvascular complications, especially before a major surgical intervention [33,34].

## Risk factors for CAN development

A poor glycaemic control and the duration of the DM diagnosis play an important role, both for triggering the physio-pathological mechanisms of CAN development (cellular destruction secondary to oxidative stress, accumulation of advanced glycosylation products, activation of the polyol dependent metabolic pathway, depletion of the nitric oxide in the microcirculatory endothelium that impairs the nerve vascularisations) and in the progression of the disease [4, 5, 33, 35, 36].

The DCCT study demonstrated, in type 1 DM patients, the favourable and substantial effect of the strict glycaemic control upon preventing diabetic microvascular complications, especially for DN. The strict glycaemic control and intensive DM treatment, together with a good metabolic control slowed down the progression of autonomic dysfunction and reduced the incidence of CAN with 53% compared to conventional therapy [37, 38]. The EDIC study, which followed-up the patients included in DCCT study, confirmed the beneficial effects of the strict therapies for hyperglycaemia. They carried a protective role upon the occurrence and progression of DN for at least 8 years after the DCCT ended. The lesser prevalence and incidence of DN was noted in the DCCT study in the lot of patients intensively treated with insulin and can be explained by a strict glycaemic control as a part of "metabolic memory". The results of these clinical studies support the need for a strict glycaemic control in type 1 DM patients in order to prevent CAN [3, 35, 39,40].

The EURODIAB study that followed type 1 DM patients for more than 8 months demonstrated that the risk for CAN development is higher in patients with poor glycaemic control, associated with arterial hypertension and various other microvascular complications [41].

A poor glycaemic control carries unfavourable long-term effects. An increase in the glycaemic levels increases the risk for diabetic complications. If the glycaemic control was inadequate in the first years of DM evolution, the incidence of complications will rise, despite an ulterior optimal control. This phenomenon is described as a "hyperglycaemic memory" or "metabolic memory" and plays a very important role, especially in type 2 DM, where patients are usually diagnosed after a variable number of years of asymptomatic evolution. At the basis of this "metabolic memory" stand epigenetic changes that arise in the silent DM period, determined by undiagnosed or untreated hyperglycaemias [42, 43].

In type 2 DM, the effects of strict glycaemic control are less conclusive, therefore CAN development is subjected to the complex interaction between the optimal glycaemic control, duration of the disease, age-dependent neuronal usage and the co-existence of cardiovascular risk factors (arterial hypertension, dyslipidaemia, obesity, smoking) [41, 44].

The UKPDS study included type 2 DM patients at the onset and evaluated the effect of the metabolic control upon the chronic complications, demonstrating that even a slight HbA1c reduction is beneficial for preventing micro- and macrovascular complications [45]. A cohort of patients included in the UKPDS study were annually monitored, both clinically and paraclinical, in order to assess the evolution of chronic complications and mortality. A decade of monitoring noted that the incidence of microvascular complications, myocardial infarction and general mortality were lower in the intensively treated group compared to conventionally treated group (at the onset of the study), although the metabolic control in the follow-up period did not differ significantly between the two groups. The UKPDS investigators named this phenomenon "metabolic inheritance". Therefore, an early optimal metabolic control for type 2 DM patients will carry beneficial long-term effects. This evidence can be attributed to the "metabolic memory" [46, 47].

Ohkubo et al. (1995) showed that type 2 DM patients with good glycaemic control on intensive insulin therapy have a lower rate of microvascular complications, including DN [48]. The VA Cooperative study revealed that there are no differences in CAN prevalence in type 2 DM patients with a strict glycaemic control compared to those without [49]. The Steno-2 study showed that the inten-

sive-aggressive intervention upon the glycaemic control, but also upon the associated cardiovascular risk factors (arterial hypertension, obesity and dyslipidaemia) reduces the CAN and microalbuminuria prevalence by up to 60% in type 2 DM patients [50].

## Cardiovascular autonomic neuropathy – an increase in cardiovascular risk and mortality

The presence of CAN in type 2 DM patients is strongly associated with the risk of malignant arrhythmias, cardiovascular events, myocardial dysfunction, silent myocardial ischemia and an increase of cardiovascular mortality [15, 51, 52].

In DM patients, due to the presence of autonomic dysfunction, the regulatory hypoglycaemic mechanisms are impaired and the patients do not perceive the typical symptoms of hypoglycaemia [53]. Hypoglycaemia can induce arrhythmias by prolonging the QT interval, by lowering the cardiovagal baroreflex function and by the means of sympathetic activation. DM and the cardiac disease accompany this phenomenon [54, 55]. In the EURODIAB study, autonomous involvement was named to be an independent risk factor for severe hypoglycaemias. Autonomous impairment contribution was demonstrated by a reduction in the secretory response of the glucagon, adrenalin and cortisol in this category of patients, therefore increasing the risk of severe hypoglycaemias [56, 57].

The ACCORD study demonstrated that in type 2 DM patients, an intensive glycaemic control, compared to standard glycaemic control, lead to an increase of general and cardiovascular mortality. Mortality was higher in the group of patients that carried a larger number of cardiovascular risk factors, and the existence of CAN at the beginning of the study doubled the mortality rate [15, 58].

Over a third of the deaths from the ACCORD study were secondary to sudden cardiac death. Therefore, the ACCORD study was the first large scale, controlled study that raised the suspicion that hypoglycaemia facilitates the onset of malignant ventricular arrhythmias [59]. A potential cause for the difference in mortality between the two groups, besides the episodes of severe hypoglycaemia, can be the existence of CAN and sensory-motor DN at the beginning of the study. The existence of CAN doubled the risk of mortality and the association between autonomic and somatic nervous impairment increases the risk of cardiovascular mortality by almost three-fold [15, 60]. A number of important conclusions were the result of the abovementioned study and they were included in the standard treatment care for type 2 DM patients:

Type 2 DM patients without cardiovascular impairment or macrovascular complications need to be encouraged to maintain an optimal glycaemic control (HbA1c≤7%);

Besides the glycaemic control, in order to decrease the risk of macrovascular complications and death, there needs to be an optimal control for the other cardiovascular risk factors (smoking, arterial hypertension, dyslipidaemia, sedentarism);

In DM patients with a long history of the disease, with advanced micro- and macrovascular complications, the level of the glycaemic control needs to be less strict compared to previous recommendations, in which the HbA1c level was indicated to be under 7% [61].

The lesson learned from the ACCORD study is that the autonomous and somatic nervous dysfunction represent risk factors for the cardiovascular disease and lead to an increase in mortality. The early identification of CAN is crucial in order to establish future treatment strategies, targeted against the mortality rate in these patients [62]. The window of opportunity for an aggressive control of the entire array of cardiovascular risk factors is defined by early diagnosis and the absence of cardiovascular disease or autonomic cardiac dysfunction [63].

## The utility of CAN diagnosis in current clinical practice

The necessity of early CAN diagnosis is due to the following:

1. Early CAN diagnosis is useful in order to establish an adequate therapeutical strategy for glycaemic control and personalised treatment [64].
2. The diagnosis of CAN has to be established before performing physical activity, and if moderate to severe CAN exists, certain physical activities will be contraindicated [6, 35].
3. The presence of CAN has to be considered as a marker for hemodynamic instability during anaesthesia, this specific lot of patients needs additional monitoring during and post-surgery [65].
4. CAN detection is necessary in order to stratify the morbidity and mortality risk. CAN is an independent risk factor for general and cardiac mortality, myocardial infarction, cardiac arrhythmias, sudden death and a progression of nephropathy [5, 6, 66, 67, 68, 69].
5. Identifying CAN patients is useful for selecting DM patients that need to be screened for coronary artery disease, in order to prevent silent myocardial infarctions, and to enhance treatment adherence [66, 70].
6. The presence of CAN can identify the DM patients that are predisposed to developing dangerous events during hypoglycaemic periods, therefore being highly useful in defining a glycaemic target (important use for assessing the risk profile of the patients, the CAN diagnosis being a contraindication for strict glycaemic control) [34].

## Conclusions

Cardiovascular autonomous dysfunction is recognised as an important cardiovascular risk factor. The importance of recognising this entity, as a predictor for increased morbidity and mortality during intensive treatment for hypergly-

caemia suggests that all type 2 DM patients need to be tested for CAN, as soon as the diagnosis is confirmed. This assessment aids the adequate management of type 2 DM patients, in order to prevent mortality.

## Authors' contribution

AM (Conceptualization; Methodology; Writing – original draft)
LB (Investigation; Writing – review & editing)
SM (Investigation; Writing – review & editing)
AB(Investigation)
AS (Conceptualization; Writing – original draft)

## Conflict of interest

None to declare.

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REVIEW

# Patient Positioning in Neurosurgery, Principles and Complications

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Patient positioning is a crucial step in neurosurgical interventions This is the responsibility of both the neurosurgeon and the anesthesiologist. Patient safety, surgeon's comfort, choosing an optimal trajectory to the lesion, reducing brain tension by facilitating venous drainage, using gravitation to maintain the lesion exposed and dynamic retraction represent general rules for correct positioning. All bony prominences must be protected by silicone padding. The head can be positioned using a horseshoe headrest or three pin skull clamp, following the general principles: avoiding elevating the head above heart more than 30 degrees, avoiding turning the head to one side more than 30 degrees and maintaining 2 to 3 finger breaths between chin and sternum. Serious complications can occur if the patient is not properly positioned so this is why great care must be paid during this step of the surgical act.

**Keywords**: positioning, complications, patient safety

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## General principles

Correctly positioning the patient is a crucial step in neurosurgical procedures. This is the responsibility of both the neurosurgeon and the anesthesiologist. Patient safety is the most important factor, this is why when positioning the patient, blood pressure and pulse oximetry should be monitored and clamping chest tubes is not allowed [1] and also we must take into consideration surgeons comfort, especially for long interventions. A correct position should provide the optimal trajectory to the lesion and whenever possible we must use positions that maintain the lesion exposed via gravity and facilitate dynamic retraction; the use of spatulas and retractors should be avoided if possible. Another key factor when positioning the patient is ensuring that venous drainage is facilitated, this way avoiding brain tension.

In this paper we aim to assess the most important principles and risk factors associated to each of the six basic positions used in neurosurgical cranial interventions: supine, lateral ("park bench"), prone (three-quarter prone) and sitting positions.

Patient positioning always starts by positioning the body first. All bony prominences must be protected by silicone padding and the body must be secured to the operating table with special padded belts. When using a lateral position or "park bench" position, lateral supports must be used, this way permitting lateral tilting of the table with the patient secured.

The operating table must fulfill the following characteristics in order to properly position the patient: it has to be a table with at least 3 sections, it has to have sliding/tilting function, and it has to have range variability between the lowest and the highest position.

## Head positioning and fixation

After securing the body, the head can be positioned. A general rule is to not elevate the head above the heart more than 30 degrees because this can lead to decreased cerebral perfusion pressure compromising cerebral blood flow. Every 2,5cm of head elevation above the heart decreases the mean arterial pressure by 2mmHg [2]. Another rule is that the distance between the chin and sternum shouldn't be less than 2 to 3 finger breaths, because hyperflexion can lead to cervical cord ischemia. The head shouldn`t be turned more than 30 degrees to one side, especially when turning it towards the dominant jugular vein.

The head can be fixed using a three pin skull clamp (Mayfield fixator) or using a horseshoe headrest. When fixing the head with the tree pin skull clamp, the imaginary line between the pins has to be under the equatorial line of the head; the pins must not interfere with the surgical field. Frontal sinuses, temporal squamous bone and venous sinuses must be avoided when inserting the pins. The risks associated to pin skull clamp fixation are bleeding, eye/scalp laceration and air embolism which can be prevented by using antibiotic ointment on the pins prior to insertion. El-Zenati et al. [3] reported one case of venous air embolism after removal of the Mayfield skull clamp to a 33 year male. The horseshoe headrest is used in trans-sphenoidal surgical approaches or for short time interventions. Long interventions can lead to pressure alopecia when using a horseshoe headrest [4].

Our experience shows that the Mayfield skull clamp is extremely versatile and use of the horseshoe headrest has limited use.

## Supine position

Supine position can be used for approaching the frontal, temporal and parietal lobes, anterior, middle and even

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the posterior cranial fossa, lateral and third ventricles and also the cervical spine for anterior approaches. The head shouldn`t be rotated more than 30 degrees to one side. More rotation can be achieved by tilting the table or by using a roll under the ipsilateral shoulder. There are three variants of the supine position (Figure 1). The horizontal position is not well tolerated by the conscious patient for long periods of time so this is why it is not recommended. The lawn-chair position is a more natural position that can be well tolerated for long periods of time (Figure 2). The patient is positioned on the table with the head and thorax slightly elevated and the hips and knees slightly flexed. A pillow must be placed under the knees and a silicone pad must be placed under the heels. Bony prominences must be padded and ulnar nerve protected. This position has the advantage that the slight elevation of the head and legs improve venous drainage of the brain and venous return. Also elevating the head and thorax improves ventilation in the dependent zones of the lungs by displacing the abdominal organs away from the diaphragm [1]. Reverse Trendelenburg position is basically a horizontal position with the head slightly elevated.

Posterior fossa lesions can be operated through an adaptation of the supine position. Awad et al. [5] described the gravity dependent supine position used for infratentorial supracerebelar lateral approaches. The patient is positioned supine with a lateral roll under the ipsilateral shoulder and the head is flexed and rotated to the contralateral side. A lazy "S" incision is made over the transverse sinus and a lateral suboccipital craniotomy is performed in standard fashion. The dimensions of the craniotomy are reduced compared to operating in prone or lateral position because there is less need for a large exposure. The dura is cut in an "U" shape and reflected over the transverse sinus. Cisterna magna is opened and brain relaxation is achieved by removing CSF. Access to the quadrigeminal cistern is now easily obtained and more CSF can be removed if needed. The cerebellum falls and good access over the top of the cerebellum or to the postero-lateral midbrain is achieved. Using this position has the advantages of operating in sitting position but venous air embolism risk is reduced and the discomfort for the neurosurgeon is minimal. It has its limitations though, this position is not adequate for midline lesions or for patients with a stiff neck.

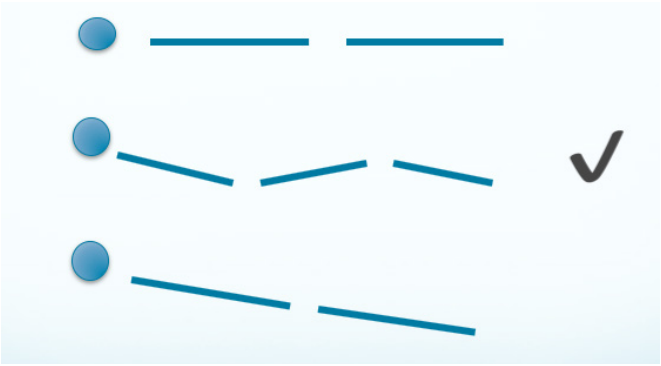

Fig. 1. Supine position variants: top-horizontal position; middle-lawn-chair position; bottom reverse Trendelenburg position

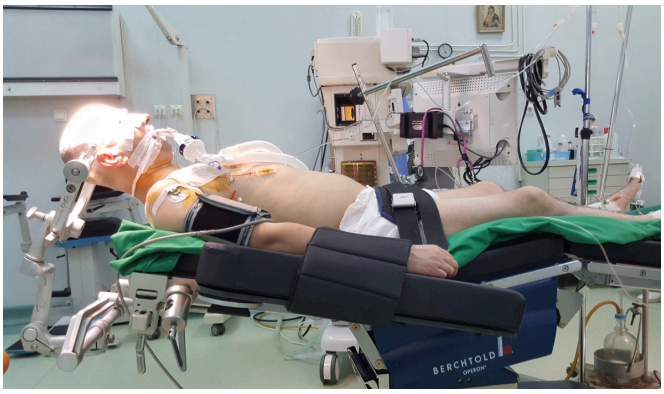


Fig. 2. The Lawn-chair position

Our experience has showed that supine position and it's minor adaptations, represented by placing a roll under one shoulder can be used to successfully operate a myriad of pathologies of anterior skull base, through unilateral or bilateral frontal or subfrontal approaches and pathologies located in the Sylvian fissure.

## Lateral position and park bench position

There are two variants of the lateral position: pure lateral and park bench position. Pure lateral position it`s mainly used for temporal area surgery, and park bench for posterior fossa lesions.

The patient is positioned with the side of the lesion upwards. When a pure lateral position (Figure 3) is needed, the long axis of the head is parallel to the ground. In park bench position (Figure 4), the head is rotated towards the shoulder contralateral to the lesion, without exceeding 30 degrees of lateral rotation. The following steps for positioning are similar for both lateral and park bench positions: a roll is placed under the contralateral upper chest, we must avoid putting it directly under the axilla because the brachial plexus and axillary vessels will be compressed; all bony prominences must be protected; the depressed arm hangs over the end of the table on an arm support or suspended with a padded string; the ipsilateral hand is placed across the thorax with the elbow in slight flexion; the ipsilateral knee is positioned in extension and the depressed knee is positioned in flexion; a pillow must be placed between the knees; lateral supports are being placed in the sternal region and in the interscapular region and afterwards the patient is secured across the pelvic region with padded strings [4].

There have been described various complications following interventions with the patients positioned in lateral or park bench, starting from pressure sores to upper limb palsy to tongue swelling or delayed airway obstruction. Koizumi et al. [6] published a case report of a 43 year old man that has developed a massive tongue swelling 13 hours after undergoing a left suboccipital craniotomy in park bench position. In this case it seems that the cause of the massive tongue swelling was a malpositioned bite block that compromised the circulation into the left side of

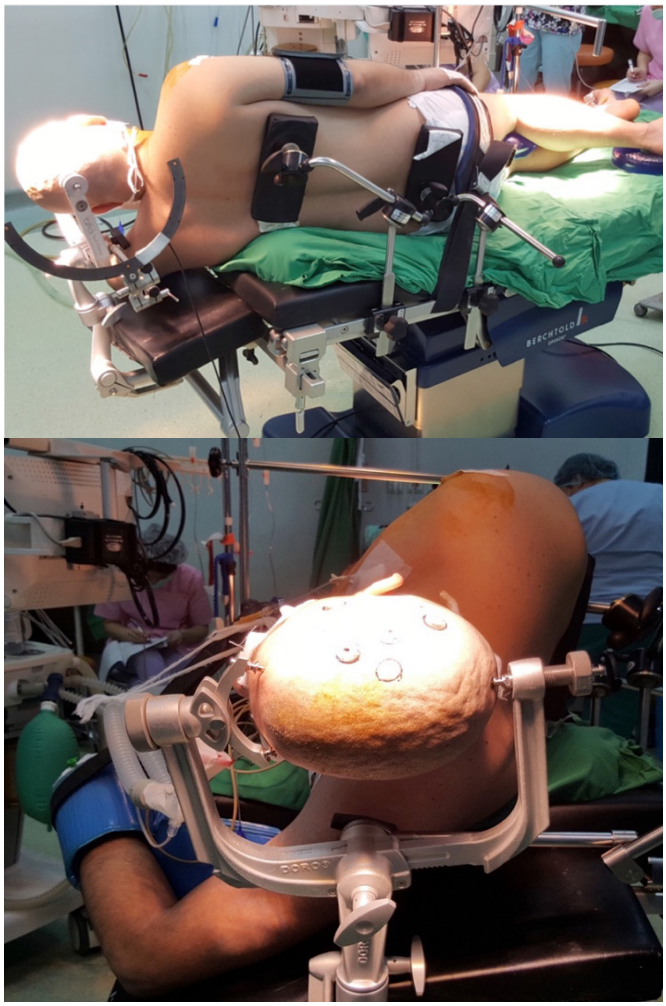

Fig. 3. The pure lateral position

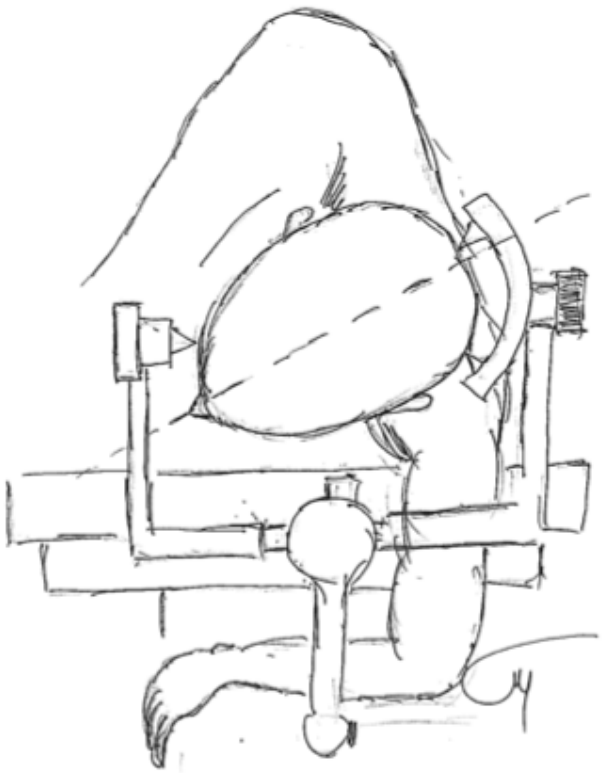

Fig. 4. The park bench position

the tongue during the intervention. The edema gradually improved after administration of intravenous steroids and no reintubation was needed. Yamaguchi et al. [7] reported two cases operated in park bench position that developed delayed airway obstruction postoperatively. They reviewed related articles written in English literature and found only five cases reported of delayed airway obstruction after craniotomy in lateral or park bench position, excluding the two cases reported by them in their case report. They concluded that excessive lateral flexion and rotation of the head (that is easily achieved especially in underweighted patients) kinks the internal jugular vein and therefore venous and lymphatic drainage from the head and neck is altered for many hours during long interventions. After the surgery is concluded, the soft tissue of the neck is reperfused causing face and neck edema that leads to airway obstruction few hours after surgery.

In our experience we have found that the lateral and park bench position are very good for treating pathologies located in the temporal, parietal or ponto-cerebellar fissure. Despite being more difficult to set up and requiring more time to do so, this time is well spent allowing for comfortable lengthy procedures for both the surgeon and the patient.

## Prone position

Prone position is being used for posterior fossa lesions, fourth and third ventricle lesions or pineal region lesions. It can also be used for posterior approaches to the cervical, thoracic and lumbar spine. The patient is intubated in supine position and afterwards it is rolled in prone position on the operating table. Two rolls should be placed under the upper part of the thorax and pelvis for releasing pressure on the abdomen. When performing spine surgery a Wilson frame can be used. A roll is placed under the shins and the knees are flexed by elevating the leg segment of the operating table. When performing cranial or upper cervical spine surgery the arms are positioned adducted along the patient and when performing thoracic or lumbar spine surgery, the arms are abducted and placed on arm boards, and the elbows are flexed; care must be exercised not to hyperextend the arms because brachial plexus injury can occur. A padded belt is placed under the fesier region to secure the patient in case afterwards the table is elevated in reverse Trendelenburg position [4].

The reverse Trendelenburg position, also known as Concorde position is mainly used for posterior fossa interventions or posterior approaches of the cervical spine. The head is then immobilized in a Mayfield head fixator and flexed, being aware to leave at least two finger breaths between the chin and sternum. A horseshoe head rest can be used for thoracic and lumbar spine interventions. Fixating the head after positioning the table in reverse Trendelenburg position prevents strain on the cervical spine. This position causes hemodynamic instability because the cardiac index and left ventricular ejection fraction decreases,

in contrast, oxygenation seems to improve because of the improved matching of ventilation-perfusion [1].

Kwee et al. [8] published a review of intraoperative and postoperative complications related to prone positioning. They analyzed 53 papers in English language literature and found 13 complications following prone position surgical interventions. The following complications were described: oropharyngeal swelling, nerve palsies (lateral femoral cutaneous nerve), postoperative vision loss, pressure sores, venous air embolism, increased intraabdominal pressure, increased bleeding, hepatic dysfunction, abdominal compartment syndrome, limb compartment syndrome, thrombosis and stroke, cardiovascular compromise and endotracheal tube dislodgement. The worst complication that can incur with the patient in prone position is cardiac arrest. A good measure of dealing with cardiac arrest is attaching the defibrillation pads before surgery. According to Nanjangud et al.[9] if a patient is diagnosed with cardiac arrest, cardio-pulmonary resuscitation must start as soon as possible with the patient in prone position, without wasting time turning the patient supine. Cardiac massage consists of manually compressing the middle portion of the thoracic spine. It seems that the systolic blood pressure generated when resuscitating a patient in prone position is higher than in supine position [10].

In our experience prone position is a very good position to do surgery in the occipital lobe, posterior fossa, craniocervical junction tumors and whole spine.

## Three quarter prone position

This is a position mainly used for posterior fossa lesions or for lesions in the parieto-occipital region. When using this position, the operative site is downwards. Three quarter prone position (Figure 5) is best suited for occipital transtentorial approaches, as described in the year 1988 by Ausman et al.[11] He described the use of this position for pineal region tumors and concluded that the risk of air embolism is reduced compared with the sitting position and that because the operative site is down, there is less need for using brain retractors.

The patient is intubated in supine position and afterwards it is rotated on the operating table. A roll is placed under the contralateral hemithorax, elevating it approximatively 15 degrees of the horizontal plane. A small roll is placed in the ipsilateral axilla. The contralateral arm is placed along the body and the ipsilateral hand is positioned behind the body. The superior leg is flexed and the inferior leg is extended. A pillow must be placed between the knees. The head is then fixed in the Mayfield device that is attached to the operating table. Usually the nose is positioned perpendicular to the ground (but the head can be rotated as much as 45 degrees) and the neck is slightly flexed. The body is secured with padded strings to the operating table. This position offers good comfort for the surgeon [4].

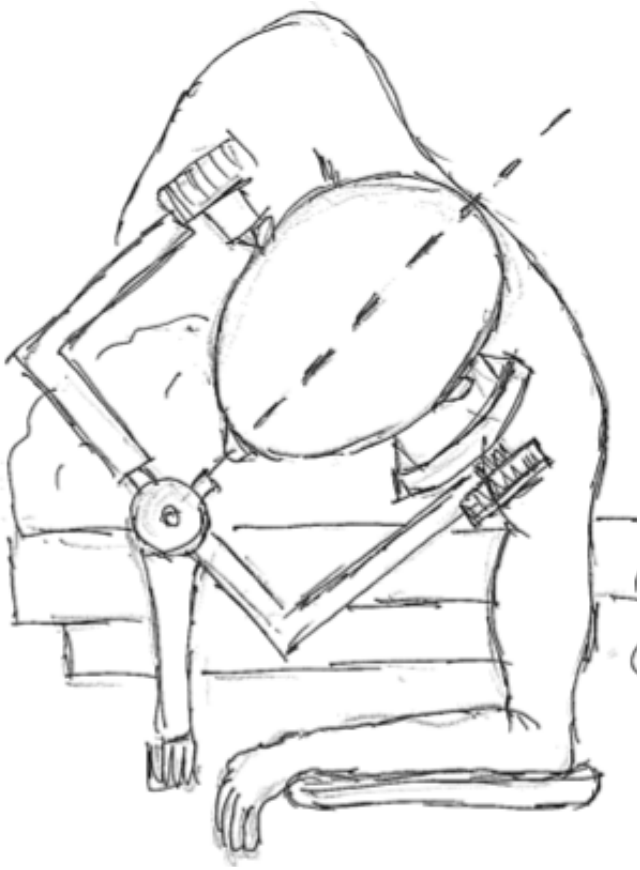

Fig. 5. The three quarter prone position

In our experience the three quarter prone position can be a good alternative to park bench in parieto-occipital of lateral posterior fossa tumors.

## Sitting position

The sitting position (Figure 6) is less used nowadays because of the increased complication ratio related to it. It is mainly used for posterior fossa lesions that are approached through the infratentorial supracerebelar approach, suboccipital transtentorial approach, retrosigmoid approach or approaches to the superior cervical spine. Also patients that need implantation of deep brain stimulators are positioned this way because brain shift is minimized in comparison to other positions [12].

The patient is intubated supine and afterwards the operating table is flexed, elevating the thorax and body of the patient in sitting position. The hips are also positioned in slight flexion and the knees should be also slightly flexed. A crossbar that attaches to the first segment of the table is positioned anterior to the patient. The head is fixed in the Mayfield head holder that is attached to the crossbar. The head is flexed until the tentorium is as parallel as possible to the ground but hyperflexion of the head should be avoided [4].

Sitting position has the advantage that the cerebellar structures are gravity-retracted, leaving a good operating corridor to the pineal gland or the superior cerebellar area.

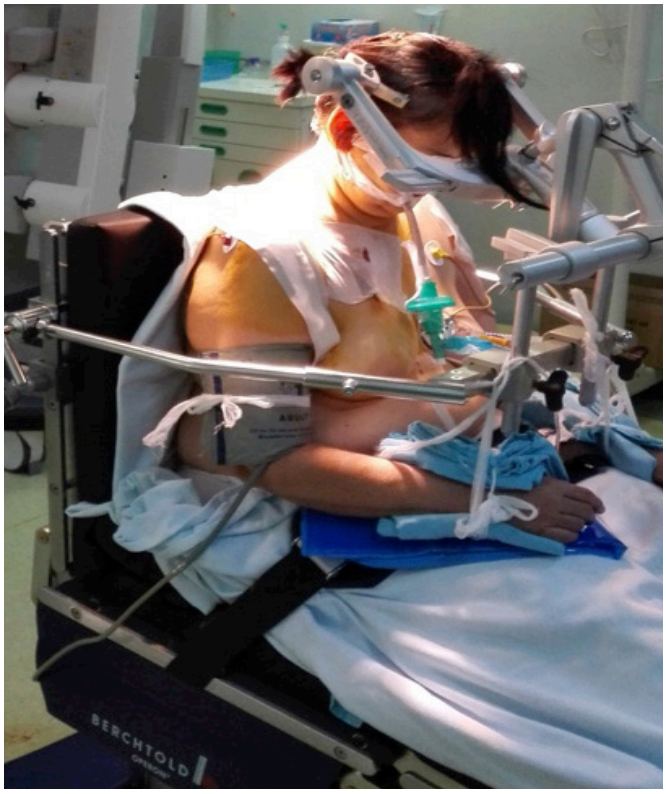

Fig. 6. The sitting position

Another advantage to sitting position is that the CSF and venous drainage improve, decreasing the cerebral pressure. The reason why this position is less used nowadays has to do with the increased risk of venous air embolism and hemodynamic instability. Dilmen et al. [13] published a retrospective study in the year 2011 of 692 cases (601 adults and 92 children) operated in sitting position and concluded that venous air embolism, diagnosed using capnography, has an occurrence of 26.3% in children and 20.4% in adults and that position induced hypotension occurs more frequently in adult population (37,6%) compared to pediatric population (18,6%). They also concluded that patients suffering of chronic obstructive pulmonary disease tolerate with great difficulty venous air embolism and recommend not using the sitting position in these patients.

Himes et al. [12] published a study of 1792 patients operated in sitting positions and reported a overall complication rate of 1,45%. The incidence of venous air embolism was 4,7%. Similarly with other studies, the incidence of venous air embolism seems to be the highest in cranial suboccipital interventions and intradural cervical spine interventions compared with cervical extradural interventions that had much lower incidence of venous air embolism. The reason why this is happening is not clear. Another complication that can appear is tension pneumocephalus that indeed in sitting position is more frequent than in other positions, but this complication frequently resolves by it's self. Subdural hematoma is a rare complication that has also been reported and the cause seems to be torn bridging veins by the mechanical displacement of the cerebellum; nevertheless tension pneumocephalus and subdural hematomas are complications that can appear in every cranial procedure. Cervical quadriplegia is a devastating complication that is specific to sitting position and can be prevented by relieving the strain on the cervical spine by properly sustaining the patient`s body, without leaving it hang by the patient`s head which is firmly placed in the Mayfield head holder; this situation, combined with position related hypotension can lead to cervical spine ischemia leading to quadriparesis or quadriplegia. Sciatic nerve injury is another complication that can appear.

Sitting position is a relative contraindication to patients that are diagnosed with patent foramen ovale because of the risk of paradoxical air embolism. Echocardiography is the screening method recommended to every patient that is a candidate for a neurosurgical intervention in sitting position [12, 13].

Despite being proved as safe we have used the sitting position just a limited number of cases, mainly for infratentorial-supracerebellar approaches. We fell that the prone position allows for similar results in other lesions of the posterior fossa whilst allowing the operating surgeon more comfort and avoiding the intraoperative complications described by placing the patient in sitting position.

## Conclusion

We can conclude that serious complications for the patient can occur if it is not properly positioned so this is why great care must be paid during this step of the surgical act. Also the surgeon`s comfort is a very important aspect that has to be taken into consideration when positioning the patient.

## Authors' contributions

AB (Conceptualization; Project administration; Supervision; Validation; Visualization)
CIH (Data curation; Investigation; Methodology; Resources; Writing – review & editing)
FT (Data curation; Formal analysis; Investigation; Methodology; Resources; Visualization; Writing – review & editing)
RC (Data curation; Formal analysis; Methodology; Project administration; Supervision; Visualization)

## Conflicts of interests

The authors of this paper state that they have no conflict of interests to disclosure.

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RESEARCH ARTICLE

# Factors Involved in the Pathogenesis of Acne and Its Psycho-Social Impact

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With acne as one of the most common and frequent conditions of young adult patients, we looked for significant associations, associated conditions with influence on the skin condition with the idea of outlining a different approach of the acne patient. With a multifactorial, multidisciplinary etiopathogeny, the purpose of this study was to highlight the factors involved in the pathogenesis of this condition and to identify those that should be taken into account when prescribing the treatment.

**Keywords**: acne, associated conditions

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## Introduction

Acne is a common condition of the pilosebaceous unit. It is considered a chronic condition, with an increased relapse rate that requires long-term care and treatment. It has a major psycho-social impact, an element that should not be forgotten considering that 80% of acne cases occur in adolescents [1]. The etiopathogeny of acne is multifactorial, not completely elucidated, but there are several elements that contribute to maintaining a chronic inflammation around the pilosebaceous unit: the excretion of sebum is increased (the involvement of androgen hormones), the appearance of hyperkeratinization which leads to the obstruction of the follicular ducts (as cause the local application of comedogenic preparations cannot be excluded), the presence of *Cutibacterium acnes*, the increase of FGFR2 (increased fibroblast growth receptor-2), and, last but not least the genes of the patients (family history). All these factors are interdependent and under immune, as well as hormonal control [2-4]. Higher sebum production has been observed in patients with acne than in the rest of the population. Triglycerides, lipoperoxides make up the composition of sebum and take part in the pathogenesis of the disease. The latter produce pro-inflammatory cytokines, whereas triglycerides are degraded by *Cutibacterium acnes*, favoring the comedogenic process and bacterial proliferation. The result of follicular hyperproliferation is the microcomedone. The infundibulm becomes hyperkeratotic with increased agglutination of keratinocytes. The follicular ostium, due to the excess of newly formed cells, will be blocked by a plug. It forces keratin, sebum, and bacteria to gather in the hair follicle. This small collection represents the microcomedone. The causes of keratinocyte hyperproliferation are varied including: low linoleic acid, androgen stimulation, dihydrotestosterone, increased interleukin-1 alpha (IL-1) activity, and the consequences of *Cutibacterium acnes*.

With a multifactorial, multidisciplinary etiopathogeny, the purpose of this study was to highlight the factors involved in the pathogenesis of this condition and to identify those that should be taken into account when prescribing the treatment.

## Material and methods

As research instrument we used an anonymous questionnaire that tracked socio-demographic aspects, hereditary-collateral history, relevant pathological personal history, the type of acne lesions, the treatment of the patient, the presence of the antibiotic treatment and its effectiveness. We investigated the use of comedogenic products or the excess use of degreasing substances. Finally, we tried to evaluate the psychological comfort of these patients. The questionnaire was distributed to a target audience, namely the patients diagnosed with the condition. The distribution of the questionnaire took place both in the online environment and in a private dermatovenerology practice. The study group included 121 patients (87 women and 34 men) with a mean age of 15 years. The most frequent age of onset of the condition was in descending order 22.3% at 14 years, 17.4% at 13 years, 16.5% at 15 years, and 11.6% at 16 years. All patients consented to the fact that their data should be published anonymously, and a signed informed consent was obtained.

Nominal variables were described as absolutes and relative frequencies (%) and the association between them was analyzed by Pearson's Chi-square test or Fisher's Exact Test. The level of statistical significance for all two-sided tests was set at $\alpha < 0.05$. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 22, Chicago, IL, USA).

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## Results

The study group included 121 patients (34 men and 87 women), with a mean age of 15 years. Patients participating in the study reported the following distribution of acne: T-zone 52.10%, cheekbones 34.4%, interscapular 22%, entire face region 19%, and presternal 9.1%. Open comedones predominated in 71.9%, closed comedones in 54.5%, cystic lesions 35.5%, and papulo-pustular lesions 55.4%. Their distribution was not uniform in the affected area, in 46.3% of cases more lesions are found and grouped unilateral compared to the contralateral side (Table I).

Most patients reported hypersecretion of sebum in the face area, encountered in the category of oily/ oily skin types - 42%, or mixed (seborrheic areas alternating with normal or dry areas) - 44%. As a result, patients frequently use face cleansing gels, 56% of them using such products on a daily basis.

Following the application of the interferential statistics tests, we found a statistically significant correlation, with $p < 0.05$ between these patients and the presence of open comedones. Another statistically significant association, with a p under 0.05 was found between the presence of closed comedones and the skin types present in the study (a majority comprising oily and mixed types). Over a quarter (26.4%) of the patients consider that they have an increased activity of exocrine sweat glands compared to the rest of the population. Regarding the family history, 58.7% have at least one relative of the first degree who suffered from this condition, and with $p = 0.004$ these patients are associated with the presence of open comedones.

To target the group of female subjects, we included a few specific questions regarding polycystic ovary syndrome, the regularity of the menstrual cycle, or the influence of menstruation on the appearance of the lesions. The results show that 29% of patients do not have menstrual cycles at regular intervals. The period before menstruation is known due to the increased number of comedones, the statistics confirming this in 50.4% of cases. In hyperandrogenism syndrome, the presence of polycystic ovaries was 16.09%, whereas 28.7% had never been under investigation for this condition. Another sign would be excess hair – hirsutism – present in 18.39% in the female group (Table II).

Subjects diagnosed with endocrine disorders were 10.7% and among the most common conditions were those involving the thyroid gland (thyroiditis, goitre, thyroid nodules, hypothyroid), breast node, adrenal disorders or previously diagnosed cases of hyperandrogenism.

After applying the interferential statistical tests and more specifically, applying the Pearson Chi-Square test, a statistically significant association was found between the presence of open comedones and disorders associated with gastric conditions (gastritis, ulcers, constipation, dyspepsia, intestinal transit disorders) with a $p < 0.05$.

At the time of completing the questionnaire, 65.3% had already used a drug treatment against acne. In these cases we found a $p = 0.008$ following the application of the Chi-Square test for the initiation of drug treatment in closed comedone type lesions. This treatment was recommended by the dermatologist (52.9%), the general practitioner (8.3%), or by friends, relatives or self-medication (17.4%). The treatment of this group varies greatly from local, topical treatments with tretinoids, or antibiotics, dermatocosmetic products, pharmacy preparations, contraceptives, oral isotretinoin, oral antibiotics, sulfur preparations.

However, a therapeutic formula stands out: the use of ointments intended exclusively for veterinary use. They contain a combination of 3 types of antibiotics, and usually 2 types of corticosteroids. Obviously, their use is not recommended by the doctor.

A percentage of 39.7 used antibiotic treatment either topically or orally. We observed a statistically significant association between the commencement of antibiotic treatment and the patients with papulo-pustular lesions. Overall, after treatment by the attending physician, either specialist or general practitioner, 57.9% of patients observed improvement in symptoms and a curative effect.

Compliance with the dermatological treatment was difficult for most patients, only 57.9% of them strictly followed the doctor's recommendations, 14% omitted administering treatment one day a week, and 5% two days a week.

Acne being a condition with a localization on an exposed area, frequently encountered in young patients, we have to take into account the psycho-social impact, 45% of the subjects considered that their daily life was affected by this condition.

## Discussions

Acne has long been treated as a condition with purely dermatological and cosmetic implications. However, some studies have focused on the association with other systemic diseases [5]. The role of androgen hormones in the pathogenesis of acne has often been studied and mentioned in the literature [6], especially in female patients, who, in 37%, had one or more signs of hyperandrogenism [7]. In

Table I. Lesion distribution

| Type of lesion | Percentage (%) |
|---|---|
| Closed comedones | 71.9 |
| Open comedones | 54.5 |
| Papulo-pustular lesions | 55.4 |
| Cystic lesions | 35.5 |
| Unilaterality* | 46.3 |

*: uneven distribution of the lesions

Table II. Distribution of endocrine symptoms

| Symptoms | Percentage (%) |
|---|---|
| Hirsutism | 18.39 |
| POS* | 16.09 |
| Other conditions** | 10.7 |

*: polycystic ovary syndrome; **: other endocrinological disorders

our study we found that 29% did not have menstrual cycles at regular intervals, 16% were diagnosed with polycystic ovary syndrome, and 18.39% had hirsutism (which indicates a hyperandrogenism syndrome), factors considered aggravating by Ewa Chlebus et al. [8]. Among the syndromes associated with this condition, we mention: POS (polycystic ovary syndrome) - where insulin resistance and hyperandrogenism are responsible for skin involvement, SAHA syndrome (seborrhea, acne, hyperandrogenism, and androgenetic alopecia) [9]. One study concludes that, in most cases, acne is not just a purely cosmetic condition and further investigation on the side of endocrine pathology is recommended [10]. Adjuvant treatment of acne using ultraviolet radiation is known, and more recently, light emitting diodes have been applied [11]. In our study, 55.4% of the patients considered sun exposure useful, which is also found in other studies [12,13]. Of or patients, 41.3% suffered from gastrointestinal disorders, and previous studies confirm a significant association with the condition in question. A study conducted on approximately 13,000 subjects of Chinese nationality found correlations between sebaceous gland condition (including acne) and constipation, gastric reflux, or bloating. A clinical success in the treatment of sebaceous gland dysfunction is the use of H2 histamine receptor blockers, which have an inhibitory effect on gastric secretion, hence the connection between the two conditions [14,15]. A controversial topic, the influence of diet as an aggravating factor, has often been debated in specialized articles, with different opinions and results. The risk factors include sweets, carbonated beverages, white bread, family history, however, the intake of milk, yogurt, cheese are considered irrelevant [16]. Our results are biased towards dairy consumption.

Regarding the use of veterinary ointments, we believe that a further study with a question specifically aimed at this would bring more data, because patients seem reluctant to admitting such usage, nevertheless this practice seems not that uncommon.

Taking into consideration that most patients reported hypersecretion of sebum in the affected areas, patients resort to frequent use of face cleansing gels. Often, an adverse effect of sebum overproduction can occur due to excessively drying of the skin by aggressive degreasing methods (alcoholic solutions, abrasive cleaning gels).

Several sources cite that this condition is genetically inherited [17], and is considered a genetic disease of the follicles prone to acne [18]. Based on our statistics we found that 58.7% have at least a first-degree relative who suffered from this condition. Although the psychological impact of acne in our country is not present in specialised publications, other countries often approach this topic. Increased risk for depressive states and anxiety in this condition has been described several times, being compared, in terms of impact, with epileptic disease. Moreover, the location of the lesions, their shape and the patient's psyche should also be taken into consideration [15,19]. Our results reflect these assertions, however, future studies should view age, shape (mild or severe), and social environment.

## Conclusion

Many patients do not seek medical attention for confirming the right diagnosis and therapeutic conduit, we underline the importance of doing so, in order to avoid the danger of self-medication. Long term use of topic corticotherapy may produce local skin atrophy, induce eczema and other well-known side effects. Being a skin pathology that seems to be easily recognized by civilians, self-medication is often the first line treatment (ranging from dermato-cosmetics to ointments intended for veterinary use), medical advice being sought only if empiric, over the counter medication does not work.

In the case of female patients, it is recommended to investigate the endocrine condition for hyperandrogenism and polycystic ovary syndrome. It is recommended to investigate all patients for digestive disorders.

Psychological guidance and counseling of these patients should be implemented when appropriate.

As the frequency of open comedones take up to 72% comedogenic products should be avoided, and, if necessary, usage of only gentle cleansing gels with moisturizing properties, underlining the importance of moisturizing agents, we would encourage that the doctor consultation will provide further support and education towards adequate skin care routine, in addition to writing a prescription, also taking phototherapy into consideration as an adjuvant treatment.

It is also recommended to inform patients about the long duration of therapy and the importance of observing the course and evolution of treatment.

## Conflict of interest

None to declare.

## Authors' contributions

SV (Conceptualization; Methodology; Supervision; Writing – review & editing)
RT (Conceptualization; Methodology; Supervision; Writing – review & editing)
TV (Conceptualization; Methodology; Supervision; Writing – review & editing)

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RESEARCH ARTICLE

# Comparative study of Clinical Characteristics in Patients with Mild and Severe Reflux Esophagitis

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**Objective**: This study aims to determine the correlation between risk factors and erosive esophagitis development. **Methods**: We conducted a retrospective observational study on a consecutive series of 19.672 patients who underwent upper gastrointestinal endoscopy between 01.01.2011-31.12.2017. A total of 3005 patients, diagnosed with erosive esophagitis, were included in the present study and stratified according to Los Angeles classification. **Results**: During the studied period we found 3005 patients with erosive esophagitis, sex ratio male to female was 1.3/1, the most common forms of esophagitis being grade A and B: 74.54% patients with esophagitis grade A, 14.80% patients with grade B; 5.29% patients were with grade C and 5.35% patients with esophagitis grade D. In severe esophagitis the male predominance was more prevalent (249 males, 71 female), with a sex ratio 3.50/1. The correlation of male gender with severe esophagitis was highly statistically significant ($p < 0.0001$, OR 2.97; 95% CI 2.25-3.91). Hiatal hernia was diagnosed in 1171 patients, the presence of large hiatal hernias, being an important predictor, with statistical significance ($p < 0.0001$, OR 3.41; 95% CI 2.22-5.21), for severe esophagitis development. Incidence of Helicobacter pylori infection was 11.51%, in the entire study group, with no statistical significant difference between patients with mild or severe esophagitis (12.02% vs 7.18%). **Conclusion**: Erosive esophagitis is a frequent disease, the most common forms being grade A and B. Male gender and the presence of hiatal hernia are the most important risk factors for erosive esophagitis development, in our study group.

**Keywords**: esophagitis, endoscopy, hiatal hernia

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## Introduction

Gastroesophageal reflux disease (GERD) is a frequent disease with a prevalence of 10-20% in western countries and with an increasing incidence in the rest of the world.[1-3] In addition to economic and medical resources involved in this chronic condition, it is accompanied by impaired quality of life and predispose the patients to develop Barrett's esophagus and esophageal adenocarcinoma. The choice of this topic was motivated by the increasing incidence of reflux pathology in our day to day work and, on the other hand, by the fact that the majority of epidemiological data are from western countries, where the prevalence of GERD and Barrett's esophagus is high and the prevalence of Helicobacter pylori infection in population is low.

The prevalence of erosive esophagitis and Barrett's esophagus is not known in central and eastern European countries, an area with high prevalence of Helicobacter pylori infection.

Many questions about the epidemiology and risk factors for erosive esophagitis remained unanswered. Therefore, this study aimed to determine the prevalence of erosive esophagitis and stratification of risk factors involved in their development.

## Methods

We performed a retrospective observational study between 01.01.2011-31.12.2017, which included 3005 diagnosed patients, diagnosed with erosive esophagitis, in the Laboratory of Endoscopy of Clinical County Emergency Hospital Târgu Mureș.

Esophagitis lesions were classified according to Los Angeles classification in 4 degrees: A, B, C, D. Patients were stratified into two groups: mild esophagitis (grade A and B) or severe esophagitis (grade C and D).

In the study were included patients with erosive esophagitis and age above 18 years. Exclusion criteria were partial or total gastrectomy, prior diagnosis of gastric or esophageal malignancy, scleroderma, liver cirrhosis and esophageal varicose veins, inconclusive pathological results and lack of consent. The study was approved by the Ethics Committee of the Hospital and a written informed consent was obtained from each patient before endoscopy. Endoscopy was performed in a standardized manner by experienced endoscopists using an Olympus Exera II instrument. Biopsies were collected to highlight the presence of Helicobacter pylori infection in each patient, 2 biopsies from the gastric antrum, 1 from angulus and 2 from the gastric body. After fixation, inclusion in paraffin and sectioning hematoxylin eosin (HE) and Giemsa stains were performed.

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Hiatal hernia was diagnosed by upper digestive endoscopy when the esophageal gastric junction was displaced 2 cm or more from the diaphragmatic hiatus. Between 2-4 cm we considered as small hiatal hernia, respectively over 4 cm as large hiatal hernia.

Data were collected with Microsoft Excel program and analyzed with Graph Pad InStat program. Categorical data analysis was conducted with the Fisher exact test or $Chi^2$ test. The level of significance was set at $p<0.05$.

## Results

Data showed that erosive esophagitis is a frequent disease (3005 patients), the most common forms of esophagitis being grade A and B: 2240 (74.54%) patients with esophagitis grade A (Los Angeles - LA), 445 (14.80%) patients with grade B esophagitis (LA), 159 (5.29%) patients with grade C esophagitis and 161 (5.35%) patients with esophagitis grade D (Figure 1).

The patients were divided into two subgroups: one group of mild esophagitis (grade A and B), 2685 patients and the group of severe esophagitis (grade C and D), 320 patients (Figure 2).

It was tracked the number of patients belonging to the two groups depending on the severity of the erosive esophagitis and observed the increased prevalence of the mild forms compared to the severe ones (Figure 3).

Analyzing the distribution of cases by gender, it was found a predominance of males 56.63% (1702), with only 43.36% (1303) females, with a sex ratio M/F-1.3/1.

Regarding the distribution of cases and severity of esophagitis, a predominance of males in mild esophagitis (1454 male, 1232 female ) was observed, while in severe esophagitis the male predominance was more prevalent (249 males, 71 female ) with a sex ratio 3.50/1. Association of male gender with severe esophagitis was statistically highly significant ($p < 0.0001$, OR 2.97; 95% CI 2.25-3.91).

The mean age was 56.29±14.19 years. In mild esophagitis the mean age was 55.78±14.05 years, lower that in the severe esophagitis group, 60.65±14.63 years.

As risk factor involved in the development of esophagitis, hiatal hernia was diagnosed in 1171 (38.96%) patients. Comparing the mild esophagitis (2685 patients) with severe esophagitis (320 patients) group, the incidence of hiatal hernia was 40.03% vs 30%, with no statistical significant difference ($p>0.05$), between the two groups. Small hiatal hernia was present in 839 (31.24%) patients with mild esophagitis and in 49 (15.31%) with severe esophagitis. The presence of small hiatal hernia was an important predictor for mild esophagitis development, with statistically significant ($p< 0.0001$) value. Also analyzing the correlation between the size of the hernia and esophagitis severity, large hiatal hernias association with severe esophagitis was highly statistically significant ($p < 0.0001$, OR 3.41; 95% CI 2.22-5.21).

Helicobacter pylori infection was detected in 11.51% (346 patients) cases from our study group, with a incidence of 12,02% (323 cases) in patients with mild esophagitis and reduced incidence of 7.18% (23 cases) in patients with severe esophageal lesions, with no statistical significant difference between the 2 groups.

We analyzed the presence of upper gastrointestinal bleeding in patients with reflux esophagitis. Upper gastrointestinal bleeding was present in 297 (9.88%) of cases with esophagitis, 185 cases in the group of mild esopha-

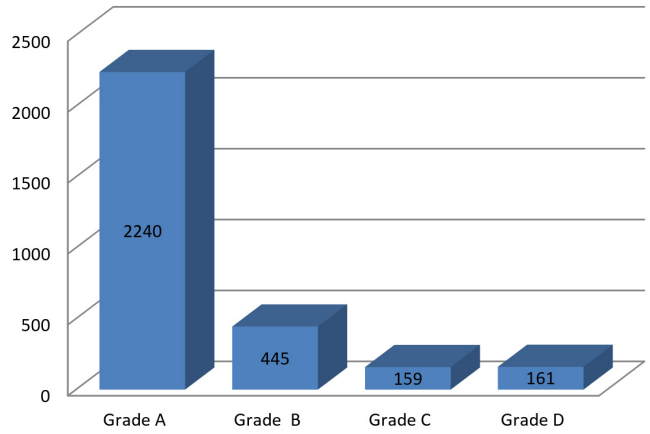


Fig. 1. Distribution of erosive esophagitis by severity

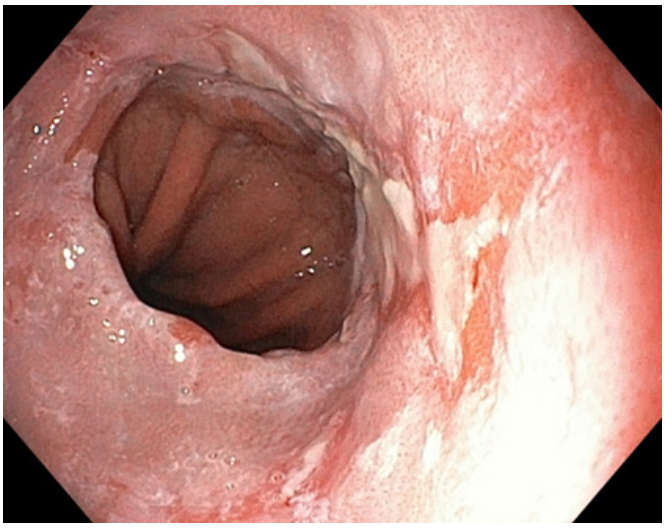

Fig. 2. Endoscopic image of severe erosive esophagitis

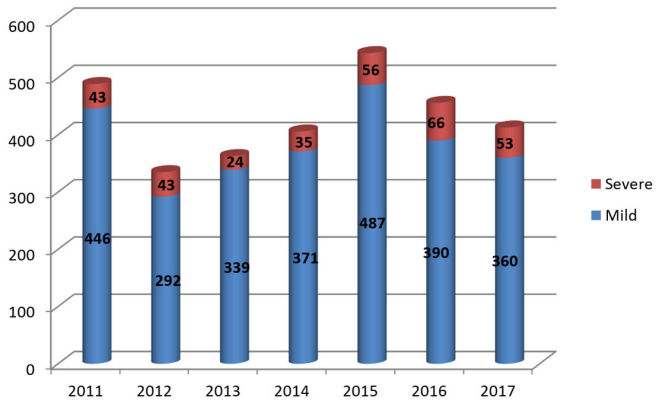


Fig. 3. Distribution of esophagitis by year

gitis and in 112 cases in the severe esophagitis group. The correlation between severe esophagitis and the presence of upper digestive hemorrhage was highly statistically significant (p <0.0001, OR 7.277; 95% CI 5.53-9.57). In patients with severe esophagitis, upper gastrointestinal bleeding was diagnosed in 96 (26.04%) male and 16 (3.12%) female patients. The upper gastrointestinal bleeding was more commonly found in male patients, with a predominance in the group of patients with severe forms p <0.0001 (OR: 2.50; 95% CI: 1.90-3.29).

## Discussion

In recent years there has been an increase in the incidence of reflux disease worldwide and of the economic impact of the disease. Although mortality from erosive esophagitis is low, there is an increase in the mortality rate, too. The variation in incidence of GERD may be related to the prevalence of Helicobacter pylori infection. The risk factors are more and more studied around the word. This study emphasizes the importance of recognition of risk factors implicated in etiology of reflux esophagitis. The novelty of this study is that shows data on a large number of patients in an area of high incidence of Helicobacter pylori infection.

Between 01.01.2011 and 31.12.2017, 19.672 upper digestive endoscopies were performed in the endoscopy laboratory. The prevalence of reflux esophagitis in the studied group was 15.27%, similar to other studies in Europe. In Europe, the prevalence of reflux esophagitis is estimated to be 8.8% -25.9%, with a tendency to increase the prevalence from southern to northern Europe [1-3]. El-Serag et al. observed a higher prevalence of reflux esophagitis in North America, compared to our study: 18.1% -27.8% [4]. The most common forms of esophagitis were grade A and B, 2240 (74.54%) patients with esophagitis grade A, 445 (14.80%) patients with grade B esophagitis, 159 (5.29%) patients with grade C esophagitis and 161 (5.35%) patients with esophagitis grade D. Kim and co-workers showed an equal distribution regarding the prevalence of Class A (LA) esophagitis: 74%, but with a higher prevalence of Class B (LA) esophagitis - 23% and with a much lower ratio of severe esophagitis: 3% [5]. The ratio of severe esophagitis is higher compared to our study in Japan: 12% [6] and in Malaysia: 20% [7]. This study shows, in concordance with other data's published, a higher prevalence of mild forms of esophagitis, compared with the severe ones. This may be explained by the high consumption of drugs like of proton pump inhibitor drugs (PPIs) and antacids in symptomatic GERD patients, drugs that alleviate symptoms and in the same time reduce the severity of esophageal lesions.

When gender distribution was analyzed, the male predominance was observed. 1702 patients (56.63%) were men and 1303 patients (43.36%) were women. Thus, it was obtained a M/F ratio of 1.30/1. Increased male involvement was evident in the group of severe esophagitis, with a M/F ratio of 3.50/1. A recent study showed male prevalence in England, similar to our study, with a M/F ratio of 1.8 / 1 in erosive esophagitis [8].

The mean age was 56.29±14.19 years. In mild esophagitis the medium age was 55.78±14.05 years, lower that in the severe esophagitis group, 60.65± 14.63 years. The same study in England reported an average age of 54 years in reflux esophagitis [8].

Hiatal hernia was present in 1171 (38.96%) of cases and absent in 1834 (61.03%) of cases. A recent study has shown that patients with hiatal hernia develop significantly more frequent erosive esophagitis compared to those without hiatal hernia: 47.5% and 24.2% (p <0.001) [9]. Similarly, in our study, we found a statistically significant correlation between the presence of large hiatal hernia and the development of severe esophagitis (p <0.0001). The relationship between Helicobacter pylori infection and reflux esophagitis in the literature is controversial. Helicobacter pylori infection was present in 346 (11.51%) cases. We did not find a statistically significant correlation between the group with mild and severe esophagitis. Rubenstein and colleagues described a negative association regarding Helicobacter pylori infection and erosive esophagitis (OR 0.63; 95% CI 0.37–1.08), without showing a correlation between infection and the presence of specific symptomatology of reflux disease (OR 0.948; 95% CI, 0.54–1.64) [10]. Another study reported the abolition of reflux disease symptomatology after bacterial eradication [11]. A prospective study showed that there is no significant difference between patients in whom Helicobacter pylori eradication has been performed or not on BRGE symptoms or erosive esophagitis [12]. A study in Asia, where the prevalence of gastric atrophy caused by Helicobacter pylori is high, showed a decrease in gastric atrophy after 5 years, which correlates with an increased incidence of erosive esophagitis [13]. In one study, which included adolescents and adults with specific reflux symptoms and pathological acid exposure, no difference in motility abnormalities was observed between patients with Helicobacter pylori and patients without. Eradication had no impact on acid exposure or esophageal motility [14].

Another interesting association included in our study was the correlation between reflux esophagitis and upper digestive bleeding. Upper digestive bleeding was present in 297 (9.88%) of cases with esophagitis. The prevalence is very similar to a study in America, which describes a ratio of 7.85%, with a higher incidence in men [15].

Our study has several limitations, being an observational study, performed on an endoscopic population, to accurately reflect the risk factors associated with reflux esophagitis. The retrospective design made it difficult to have all history of the patients and other risk factors like obesity and concomitant medication. Additionally, this study was conducted at only one center, which may limit the generalization. Prospective studies are needed to better define a true association between the studied risk factors and reflux esophagitis.

## Conclusion

Erosive esophagitis is a frequent disease, the most common forms being grade A and B. In our study, male gender and hiatal hernia are important risk factors for developing severe erosive esophagitis.

## Conflicts of interest

The authors have no financial conflicts of interest.

## Authors' contribution

MM (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Validation; Visualization; Writing – original draft; Writing – review & editing)
SMB (Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Writing – original draft; Writing – review & editing)
MP (Formal analysis; Investigation; Supervision; Visualization; Writing – review & editing)
RO (Data curation; Investigation; Project administration; Writing – original draft)
SM (Data curation; Investigation; Resources)
IM (Conceptualization; Data curation; Formal analysis; Investigation; Supervision; Visualization; Writing – review & editing)

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RESEARCH ARTICLE

# Thin layer chromatographic compatibility study in preformulation of new transdermal therapeutic systems

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**Objective**: The compatibility of four binary active substances combinations adapalene – levofloxacin (ADP-LFX), adapalene – miconazole nitrate (ADP-MCZ), levofloxacin – meloxicam (LFX-MLX) and levofloxacin – miconazole nitrate (LFX-MCZ) was analysed to be comprised in new transdermal therapeutic systems. Also, the compatibility of selected active substances and four polymeric excipients (hydroxypropyl methylcellulose - HPMC 15000, hydroxypropyl methylcellulose - HPMC E5, ethyl cellulose - EC 10, and hydroxyethyl cellulose – HEC) was studied. **Methods**: Thin layer chromatographic method (TLC) and four selected mobile phases were used. On the plate (*in situ*) were obtained the binary combinations (active substances and active substance-polymer). **Results**: A good compatibility of ADP-LFX was found using ammonia : methanol : acetonitrile : methylene chloride 2:4:1:4 mobile phase. Using chloroform : acetone : glacial acetic acid 34:4:3 on the chromatogram of ADP-MCZ, only ADP spots appeared but without changes in the shape of the spots and $R_f$ values. Any modifications of LFX and MLX spots (from LFX-MLX mixture) had been observed using toluene : glacial acetic acid : methanol 11:1:0.5 mobile phase, although LFX spots have remained on the baseline. Only LFX spots were visible from LFX-MLX and LFX-MCZ mixtures (ammonia : methanol : acetonitrile : methylene chloride 2:4:1:4 mobile phase). Distinctive spots were observed for ADP, LFX and MLX with variable results from no chemical interactions to limited chemical interactions when the compatibility with polymers was verified. **Conclusions**: ADP-LFX and LFX-MLX mixtures were found to be compatible. ADP with HPMC polymers and LFX with HPMC E5 and HEC had presented excellent compatibility; for the other binary combinations, different analytical methods will be necessary.

**Keywords**: TLC, adapalene, levofloxacin, meloxicam, miconazole nitrate

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## Introduction

Chromatographic methods are used in preformulation studies, one of the simplest being thin layer chromatography (TLC) method. TLC is appropriate to determine the stability and compatibility of the compounds in a pharmaceutical formulation. The mixture of the selected substances will have to provide on the chromatogram identical spots with the individual compounds if there are no interactions between them [1].

In this paper, the compatibility between four active substances – adapalene (ADP), levofloxacin (LFX), meloxicam (MLX) and miconazole nitrate (MCZ) was pre-evaluated by TLC method, subject to the analysis four binary mixtures: ADP – LFX, ADP – MCZ, LFX – MLX, LFX – MCZ, as potential combinations in formulations of new transdermal therapeutic systems (TTSs). Nowadays, the development of new TTSs formula is increasing [2, 3]. Thereby, the use of binary mixtures of active substances became a new challenge in the pharmaceutical field (Figure 1).

In dermatology, the four selected active substances are used topically or systemically as valuable therapeutic compounds. The association of a retinoid (ADP) and a fluoroquinolone (LFX) pursuits the join of combining the anti-inflammatory and antibiotic effects, as the previous study was proving the efficiency of other similar combinations [4-6], LFX being active as well in topical applications [7-10]. The association of ADP with MCZ also could have therapeutic potential, MCZ being considered beneficial in the treatment of acne, both individually and in various combinations [11-13]. Co-administration of a fluoroquinolone (e.g. LFX) with a non-steroidal anti-inflammatory compound (MLX) or an antimycotic (MCZ) could be beneficial in complex therapy [14-16].

In addition, it has been studied the compatibility of selected active substances with a series of excipients as hydroxypropylmethylcellulose (HPMC) type E5 and 15000, ethyl cellulose type 10 (EC 10), and hydroxyethyl cellulose (HEC). These previously selected excipients as TTSs matrix-forming polymers have the advantage of forming gels in water from which flexible matrices can be obtained by evaporating the water to gentle heating.

## Methods

### Apparatus and reagents

A CAMAG chromatographic system (Camag, Switzerland) has been used: Nanomat 4 and capillary dispenser, dispenser magazine and capillary pipettes 2.0 μL, develop-

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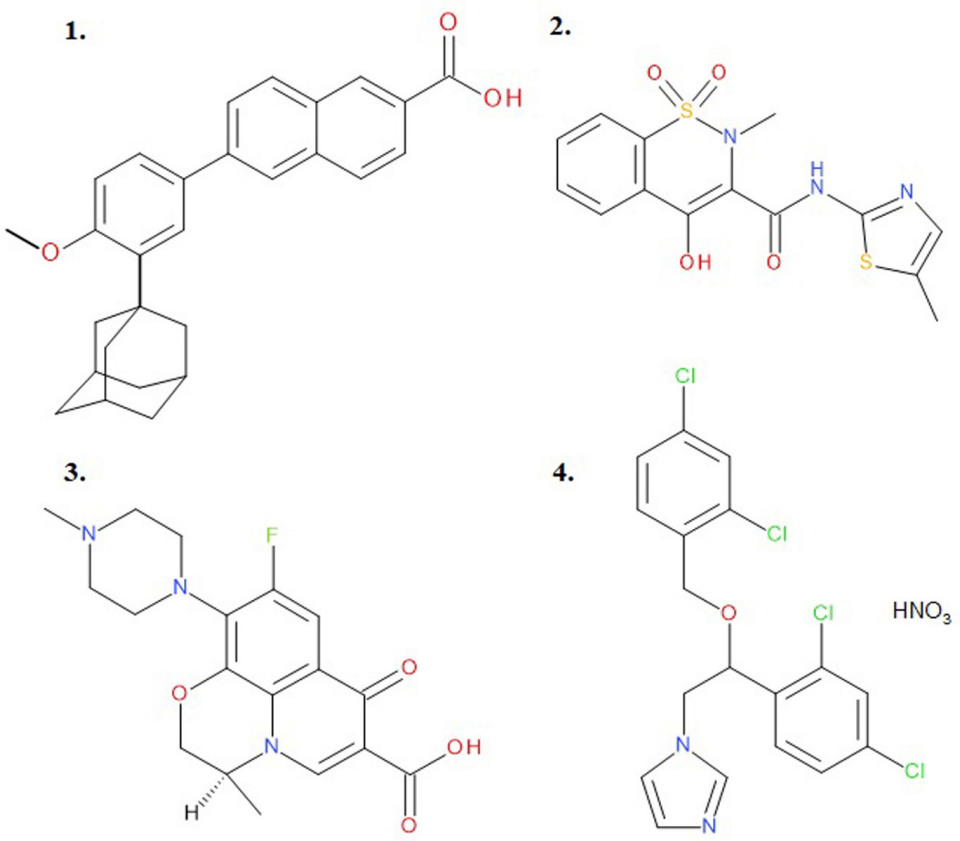


Fig. 1. The chemical structures of selected compounds: 1) ADP, 2) MLX, 3) LFX, and 4) MCZ

ing twin trough chamber for plates 20 x 20 cm, with glass lid, CAMAG dual wavelength UV lamp and a viewing box (two wavelengths, 254 and 366 nm). TLC silica gel 60 $F_{254}$ and silica gel G (aluminium sheets 20 x 20 cm) (Merck, Germany) has been used as the stationary phase.

The used standard substances and reagents were obtained as follows ADP, LFX and MCZ from Sigma Aldrich (USA), and MLX from Techno Drugs & Intermediates Ltd (India); the other used solvents were acetonitrile, dioxane, methanol GR p.a. (Lach - Ner, Czech Republic), glacial acetic acid, acetone, methylene chloride (Chimopar Bucureşti, Romania), 25% ammonia (Microchim, Romania), chloroform (Chemical, Romania), toluene (Reactivul Bucureşti, România). Polymers were obtained as follows: HPMC E5 from Dow Chemical Co., Midland, USA, HPMC 15000 from Shin-Etsu Chemical Co, Ltd Tokyo, Japan, EC 10 and HEC from Sigma Aldrich Co., Germany.

Stock solutions were prepared in methanol (1 mg/mL concentration). The control solutions were obtained from stock solutions by dilution in an optimal ratio depending on the intensity of the preliminarily obtained spots.

Samples (2.0 µL) were applied using Nanomat 4 device. The distances between the spots were set at 15 mm, and the length of the edge of the TLC plate was set at 20 mm. After the TLC plates were dried at room temperature, were developed in a chamber previously saturated with mobile phase vapour for 30 min. The ascending mode at room temperature was used (22±2°C) until the solvent front reached 15 cm distance. Further, the plates were dried 15 min in air, and the spots were revealed using the dual-wavelength UV lamp (254/366 nm) or exposing the plate to iodine vapours.

## Results

***Selection of mobile phases.*** Several mobile phases have been tested, taking into consideration appropriate TLC methods for our compounds, LFX [17-19], MLX [20-22], and MCZ [23-24]. From the best of our knowledge, there is not any described method dealing with TLC determination, nor quantification of ADP in the scientific literature.

The selected mobile phases are presented in Table I.

The elution power of the mobile phases was calculated according to the following formula [25]:

$$\varepsilon^{\circ}_{\text{fază mobilă}} = (\%_{\text{solvent A}} \bullet \varepsilon^{\circ}_{\text{solvent A}})/100 + (\%_{\text{solvent B}} \bullet \varepsilon^{\circ}_{\text{solvent B}})/100 + \ldots (\%_{\text{solvent X}} \bullet \varepsilon^{\circ}_{\text{solvent X}})/100$$

The detection was noticed using UV light at 254 nm and 366 nm, as all selected compounds exhibited good adsorption, except MCZ (Table II).

The control solutions of two active substances were prepared and spotted separately on the baseline at 2 cm from the edge of the plate. Their binary mixture was obtained *in situ* on the baseline. In the same way, the spots were applied for each drug and the four polymers. The $R_f$ values are presented in Table III. The retention parameter $\mathbf{R_M}$ was calculated, where $\mathbf{R_M = \log(1/R_f - 1)}$, which shows a linear relationship between the chromatographic and analytical

Table I. Mobile phases and their elution power values, and tested *in situ* binary mixtures (Ref. – references).

| No. | Mobile phase composition and solvents ratio | ε° | Analysed binary mixtures (in situ) | Ref. |
|---|---|---|---|---|
| 1 | toluene : glacial acetic acid : methanol 11:1:0.5 (v/v/v) | 0.37 | LFX-MLX<br>ADP-(polymer)<br>MLX-(polymer) | [20] |
| 2 | chloroform : acetone : glacial acetic acid 34:4:3 (v/v/v) | 0.46 | ADP-MCZ<br>LFX-MCZ | [23] |
| 3 | ammonia : methanol : acetonitrile : methylene chloride 2:4:1:4 (v/v/v/v) | 0.74 | ADP-LFX<br>LFX-MCZ<br>LFX-MLX<br>LFX-(polymer) | [19] |
| 4 | ammonium acetate : dioxane: methanol 20:40:40 (v/v/v) | 0.80 | ADP-MCZ<br>LFX-MCZ<br>MCZ-(polymer) | [24] |

Table II. Appearance of the active substances' spots at 254 și 366 nm.

| Compound | Wavelength | |
|---|---|---|
| | 254 nm | 366 nm |
| MLX | purple-tinted | light blue with low luminescence |
| MCZ | very light purple, with low luminescence | - |
| LFX | purple | light blue with strong luminescence |
| ADP | purple | intense purple with strong luminescence |

properties [25], respectively, the increased value of $R_f$ are correlated with decreasing value of $R_M$ (Table III).

***Compatibility study between ADP and LFX.*** The selected no. 3 mobile phase was used. The chromatogram shows distinctive spots for control solutions and the binary mixture obtained *in situ* (Figure 2).

***Compatibility between ADP and MCZ.*** For this purpose, the selected no. 2 mobile phase was used. The chromatogram shows distinctive spots only for ADP (obtained with the control solution and *in situ* mixture) with the same value of $R_f$ (Figure 3, scheme b).

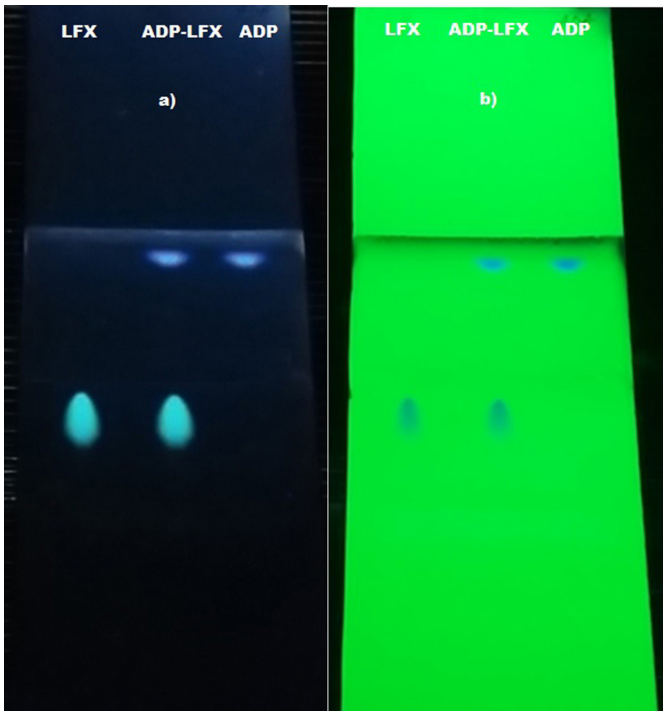


Fig 2. LFX, ADP-LFX, and ADP spots on the plate; wavelength a) 366 nm, b) 254 nm; no. 3 mobile phase

Table III. Obtained $R_f$ values using the selected mobile phases.

| Mobile phase | Active substance (from control solutions and mixtures obtained *in situ*) | $R_f$ | $R_M$ | Observations |
|---|---|---|---|---|
| No.1 | MLX (control solution) | 0.56 | -0.10 | |
| | MLX (from LFX-MLX) | 0.56 | -0.10 | Same $R_f$ value with control solution |
| | LFX (control solution) | - | - | Spot on the baseline |
| | LFX (from LFX-MLX) | - | - | Spot on the baseline |
| No. 2 | MCZ (control solution) | - | - | No spot at both wavelengths |
| | MCZ (from LFX-MCZ) | - | - | No spot at both wavelengths |
| | LFX (control solution) | - | - | Spot on the baseline |
| | LFX (from LFX-MLX) | - | - | Spot on the baseline |
| | ADP (control solution) | 0.95 | -1.27 | |
| | ADP (from ADP-MCZ) | 0.95 | -1.27 | Same $R_f$ value with control solution |
| No. 3 | LFX (control solution) | 0.62 | -0.21 | |
| | LFX (from LFX-MLX) | 0.62 | -0.21 | Same $R_f$ value with control solution |
| | LFX (from LFX-MCZ) | 0.62 | -0.21 | Same $R_f$ value with control solution |
| | ADP (control solution) | 0.94 | -1.19 | |
| | ADP (from ADP-LFX) | 0.94 | -1.19 | Same $R_f$ value with control solution |
| | MLX (control solution) | - | - | No spot at both wavelengths |
| | MLX (from LFX-MLX) | - | - | No spot at both wavelengths |

***Compatibility between LFX and MLX.*** The chromatogram shows distinctive spots of LFX and MLX obtained with control solutions and *in situ* binary mixture at 254 nm; to note that the LFX spots have not migrated from the base line (Figure 4) using mobile phase no. 1.

***Compatibility between LFX and MCZ.*** The chromatogram shows two distinctive spots only for LFX (from control solution and mixture) with the same value of $R_f$. Neither MLX or MCZ showed any spot at 366 nm (Figure 5) or 254 nm using mobile phase no. 3.

Compatibility of the active substances and polymers has been studied with the appropriate mobile phases, and the result has been comprised of Table IV. For MCZ, the results have been inconclusive with the mobile phase no. 2 (no visible spots on the plate at the two wavelengths). Thus, another mobile phase was tested: ammonium acetate R: dioxane: methanol 20:40:40 (v/v/v). Very pale spots at the reaction with iodine vapours and slight modification of MCZ $R_f$ values were revealed.

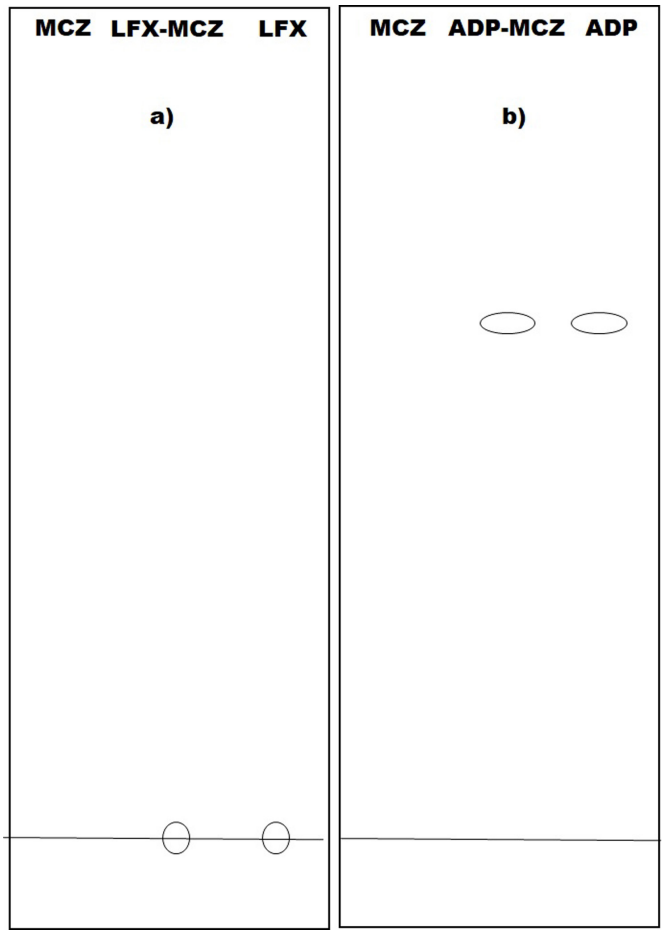


Fig. 3. Scheme of a) MCZ, LFX-MCZ, and MCZ spots on the plate; b) MCZ, ADP-MCZ, and MCZ spots on the plate; wavelength 254 nm; no. 2 mobile phase

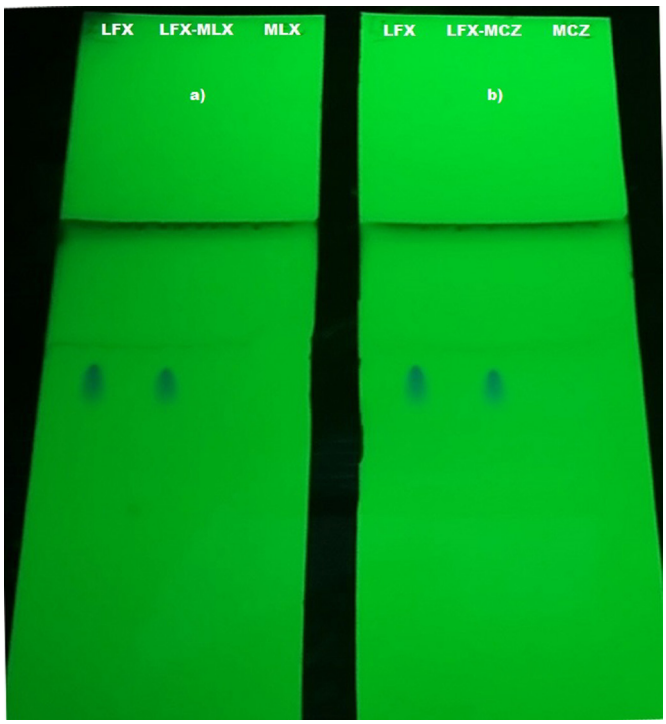


Fig. 5. a) LFX, LFX-MLX, and MLX spots on the plate; b) LFX, LFX-MCZ, and MCZ spots on the plate; wavelength 254 nm; no. 3 mobile phase


Fig. 4. MLX, LFX-MLX, and LFX spots on the plate; wavelength 366 nm; no.1 mobile phase

***Compatibility of the active substances and polymers.*** The results of the compatibility study between the ADP, LFX and MLX are comprised in Table IV and Figure 6. Probably, due to the particular solubility of MCZ, the results were inconclusive regarding interactions with the other active substances using the mobile phase no. 2 and no. 4. In the no. 4 mobile phase, the obtained spots were very pale; small interactions with polymers occurred. The

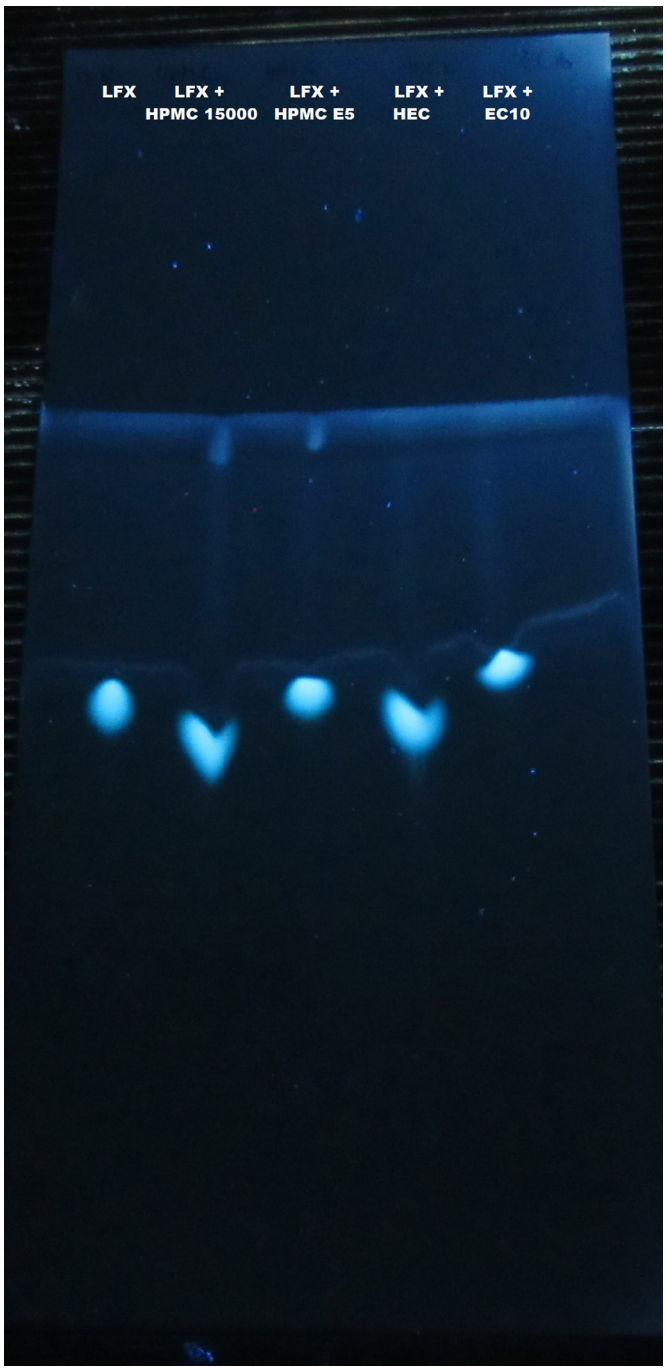


Fig. 6. LFX and in situ mixtures of LFX and polymers spots on the plate; wavelength 366 nm; no. 3 mobile phase

compatibility study of MCZ and selected polymers will be performed through other analytical methods.

## Discussion

The analysis of obtained plates was carried out and discussed as follow:

Compatibility between ADP and LFX was tested through the TLC system using no. 3 mobile phase (Table III). This mobile phase has a 0.74 elution power and forces ADP to migrate to the top of the plate as a consequence of ADP solubility. ADP is soluble in polar aprotic solvents (tetrahydrofuran, dimethylsulphoxide and dimethylformamide), sparingly soluble in protic ethanol, and practically insoluble in water (polar protic solvents) [26, 27]. The no. 3 mobile phase contains acetonitrile, another polar aprotic solvent appropriate to be used on HPLC systems for ADP analysis [28-29]. ADP and LFX spots migrated with the mobile phase with different $R_f$ values (Table III); it is clear that there is no incompatibility between the two compounds. Spots obtained from the mixture were identical to the controls and were visible at both wavelengths (Figure 2).

Though European Pharmacopoeia offers an identification method for MCZ, this was not appropriate for our compatibility experiment. Thus, no. 2 mobile phase was used (Table III) to study possible interactions between ADP and MCZ. The $R_f$ value of ADP spot obtained from the mixture was identical to the $R_f$ value of the control spot ($R_f$ 0.95) (Figure 3) and could be viewed at both wavelengths. The MCZ spot was not visible at either of the two wavelengths but seemed to be clear that ADP spot has not been influenced. Apparently, no chemical interference occurred between the two compounds. In the next stage, it will be necessary to use other analysis methods to check the compatibility between the ADP and MCZ.

No. 1 mobile phase was used for testing the compatibility between LFX and MLX (Table III). The eluent was very appropriate for MLX ($R_f$ 0.56). Instead, LFX spot has not migrated with the solvents and has remained on the baseline (Figure 4). On the no. 3 mobile phase the MLX spot was not visible, but the LFX spot has migrated with the mobile phase. The obtained $R_f$ value of LFX spot from the mixture was the same with the $R_f$ value of the control spot ($R_f$ 0.62) (Figure 5). LFX spots were visible at both wavelengths. So, the two compounds seem to be compatible but will require other complementary methods to prove it.

Compatibility between LFX and MCZ was tested using no. 3 mobile phase (Table III). The obtained $R_f$ value of LFX spot from the mixture was the same with the $R_f$ value of the control spot ($R_f$ 0.62) (Figure 5). LFX spots were visible at both wavelengths. So, the two compounds seem to be compatible, similar to the evaluation of compatibility between LFX and MLX.

The obtained results of the compatibility study of the four binary active substances and their binary combination with four selected polymers are comprised in Table V. The compatibility study of MCZ and selected polymers will be performed through other appropriate methods.

In the future, to collect the best compatibility data, more complementary methods will be necessary, such as spectroscopic methods and thermal analysis [30].

## Conclusions

The obtained *in situ* ADP-LFX and LFX-MLX mixtures were found to be compatible. ADP-MCZ and LFX-MCZ mixtures require more specific analytical methods. ADP with HPMC polymers combinations and LFX with HPMC E5 and HEC combinations presented excellent compatibility by TLC method. Also, concomitant use of

**Table IV. Results of the compatibility study between ADP, LFX and MLX and the four selected polymers (HPMC 15000, HPMC E5, HEC, and EC10).**

| Mobile phase | Compounds (from control solutions and mixtures obtained in situ) | $R_f$ | $R_M$ | Observations (comparisons to control solutions) |
|---|---|---|---|---|
| No. 1 | ADP (control solution) | 0.58 | -0.14 | |
| | ADP (from the mixture with HPMC 15000) | 0.57 | -0.12 | a slight decrease in $R_f$ values |
| | ADP (from the mixture with HPMC E5) | 0.57 | -0.12 | |
| | ADP (from the mixture with HEC) | 0.56 | -0.10 | |
| | ADP (from the mixture with EC10) | 0.55 | -0.08 | |
| No. 3 | LFX (control solution) | 0.59 | -0.15 | |
| | LFX (from the mixture with HPMC 15000) | 0.52 | -0.03 | a decrease in $R_f$ value |
| | LFX (from the mixture with HPMC E5) | 0.60 | -0.17 | |
| | LFX (from the mixture with HEC) | 0.59 | -0.15 | same $R_f$ value with control solution |
| | LFX (from the mixture with EC10) | 0.63 | -0.23 | a slight increase in $R_f$ value |
| No. 1 | MLX (control solution) | 0.57 | -0.12 | |
| | MLX (from the mixture with HPMC 15000) | 0.51 | -0.01 | |
| | MLX (from the mixture with HPMC E5) | 0.52 | -0.03 | |
| | MLX (from the mixture with HEC) | 0.51 | -0.01 | |
| | MLX (from the mixture with EC10) | 0.53 | -0.05 | |

**Table V. The result of the compatibility study; detection at 254 nm, 366 nm; *expose to iodine vapour**

| AS 1 | AS 2 Mobile phase | ADP | LFX | MLX | MCZ | HPMC 15000 | HPMC E5 | HEC | EC 10 |
|---|---|---|---|---|---|---|---|---|---|
| ADP | No. 1 | x | x | x | x | MI | MI | MI | MI |
| LFX | No. 3 | NI | x | NI | NI | SI | SI | MI | MI |
| MLX | No. 1 | NI | NI | x | x | MI | MI | MI | MI |
| MCZ | No. 2 | NI | NI | x | x | x | x | x | x |
| | No. 4 * | IC | IC | x | x | MI | MI | MI | MI |

AS – Active substance, No interactions – NI, Minor interactions – MI, Strong interactions – SI, IC – Inconclusive Chromatogram, x – no determination

several spectroscopic and thermal methods will allow a better understanding of physicochemical drug-drug and drug-polymer interactions and will be helpful in the preformulation stage of TTSs.

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## Authors' contribution

OLM (Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft)
DNB (Data curation; Formal analysis; Methodology; Writing – original draft)
NT (Conceptualization; Methodology; Supervision; Validation; Writing – original draft)
AR (Conceptualization; Methodology; Project administration; Supervision; Validation; Writing – review & editing)

## Conflict of interest

None to declare.

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RESEARCH ARTICLE

# The relative bioavailability of two formulations containing 10 mg Dapagliflozin assessed under fasting conditions in a randomized crossover study in healthy Caucasian subjects

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**Objective**: The aim of the present study was to evaluate the relative bioavailability of two formulations containing 10 mg dapagliflozin in healthy Caucasian subjects under fasting conditions. **Materials and Methods**: Forty-eight healthy Caucasian subjects were enrolled in a single-dose, crossover, balanced, open label, randomized clinical trial, with two treatment, two periods and two sequences. The wash-out period was of 7 days and thirty-eight subjects completed both study periods. Each subject received a single dose of 10 mg dapagliflozin as the reference product Farxiga® (AstraZeneca Pharmaceuticals LP, USA) and the test product developed by Sun Pharmaceutical Industries, India. Dapagliflozin plasma levels were determined from blood samples collected in both study periods before and after dosing until 48 hours by using a validated LC-MS/MS method. For pharmacokinetic analysis of data, the non-compartmental method was used (Phoenix® WinNonlin 6.3). The statistical analysis was performed by SAS software 9.1.3 for the logarithmically transformed values of maximum plasma concentration and area under the curve. **Results**: The 90% confidence intervals for the evaluated pharmacokinetic parameters were found to be in the accepted interval for bioequivalence (80.00-125.00%). **Conclusion**: The 10 mg dapagliflozin immediate release tablet newly developed by Sun Pharmaceutical Industries, India, is bioequivalent with the reference product Farxiga® under fasted state of the subjects.

**Keywords**: dapagliflozin, bioequivalence trial, Caucasian subjects, fasted state

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## Introduction

Bioequivalence and bioavailability studies are fundamental as their purpose is to establish if a newly developed formulation (test formulation) can be interchangeable with a formulation that is already authorized for marketing (reference formulation) [1,2].

In order to establish that the new tested formulation is safe and has the same efficacy as the reference product the manufacturer has to consider a bioequivalence study in which both products are compared after being administered to healthy adult human subjects [3].

The plasma concentration time curve is used to assess the bioavailability of two products and is usually correlated with the rate and extent of absorption. The most important pharmacokinetic parameters that describe the absorption proportion of the active ingredient from the pharmaceutical form are area under the curve (AUC), maximum plasma concentration ($C_{max}$), and time to reach $C_{max}$ ($t_{max}$) [4].

The generic products that are proved to have the same qualitative and quantitative composition as the original product are gaining a substantial part of the pharmaceutical market as their substitution with the reference product reduces the costs of treatment for patients [1,3,4].

The bioequivalence study should have a standardized design and subjects included in the study should undergo the same conditions, except the two formulations that are compared, in order to reduce the variability of any factors involved such as food/water intake, exercise, time of administration of the investigational medicinal product (IMP) etc [5,6].

The incidence of Diabetes Mellitus (DM) has increased during the last century and became the most common metabolic condition in the world. Based on the clinical data from International Diabetes Federation in 2017 more than 425 million adults worldwide were known to be diagnosed with diabetes and the number is believed to increase up to more than 629 million until 2045 [7,8].

Type 2 diabetes mellitus (T2DM) is found to be the most common among diabetic patients. The specialists describe eight causes that contribute to the pathophysiology of T2DM, namely decreased insulin secretion, increased glucose reabsorption, increased hepatic glucose production, increased lipolysis, increased glucagon secretion, de-

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creased glucose uptake, neurotransmitter dysfunction and decreased incretin effect [9,10]. Therefore, the therapy should target one or more of these metabolic defects and consider at the same time the patient's needs, by developing a patients-centered treatment scheme [8].

Dapagliflozin is a highly selective sodium-glucose cotransporter 2 (SGLT2) inhibitor , administered via oral route [11]. First of his class, dapagliflozin reduces hyperglycemia by inhibiting SGLT2 which is located in the proximal tubule of kidneys and responsible for the reabsorption of glucose [12]. By inhibiting the reabsorption of glucose, the elimination of glucose in the urine increases. Dapagliflozin has proved its benefits in patients diagnosed with T2DM who cannot tolerate the treatment with metformin and, since March 2019, in patients suffering from type 1 diabetes with BMI ≥ 27 kg/m$^2$ when treatment with insulin does not provide an adequate control of glycaemia [13].

The patients suffering from DM, either type 1 or 2, are generally facing other associated health problems such as dyslipidemia, obesity, high blood pressure or cardiovascular diseases. Therefore, the treatment usually consists of two or more drugs that patients should take daily. The more medication is added to the therapeutic scheme, the more the costs increase [8,9].

On the other hand, a good approach in the treatment of DM consists of a healthy lifestyle that combines diet with exercise and avoids smoking or alcohol intake [8].

Combined with diet and physical exercise dapagliflozin helps to reduce the weight of patients, factor that can highly influence the outcome of the treatment when T2DM is associated with obesity or other cardiovascular risks [10,14].

The objective of this study was to determine and compare the oral bioavailability and pharmacokinetics of two different immediate release formulations containing 10 mg dapagliflozin, after administration in healthy adult human subjects under fasting conditions.

## Materials and Methods

### Subjects

The clinical bioequivalence study was conducted respecting all the principles defined in the Declaration of Helsinki (Brazil 2013) and the principles from the Guidelines for Good Clinical Practice ICH E6(R2) (CPMP/ICH/135/95). Furthermore, it was applied the draft guidance on generic drug development for Dapagliflozin tablets enunciated by the U.S. Food and Drug Administration [12]. Before initiating the study, the National Agency of Medicine and Medical Devices, Romania, and Bioethics National Committee of the Medicines and Medical Devices, Romania, approved the clinical study protocol. The clinical trial was performed at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA-Sun Pharma, Romania.

In order to determine the number of subjects needed for this clinical study, the sample size estimation was calculated based on the available in-house and literature data on dapagliflozin using SAS® system for Windows release 9.1.3, SAS Institute Inc., USA. Thus, it was taken into consideration a Test/Reference ratio of 90-110% and an intra-subject coefficient of variation (CV) of approximately 20%. Therefore, to yield a power of 80% to show bioequivalence of the test and reference products, under bioequivalence assumptions, a number of 36 subjects resulted to be sufficient for enrollment. Nonetheless, possible dropouts of the subjects and/or withdrawals were considered, thus 48 subjects were eventually enrolled in this study.

All subjects included in the study had signed the written informed consent and they were carefully instructed with regard to the details of study such as periods schedule, rights, restrictive and obligations and possible side effects of administered drugs.

The complete study program was communicated to volunteers at the time of consent. All subjects included in the study underwent some standardized screening procedures that consisted of physical examination, vital signs measurements (axillary body temperature, sitting blood pressure and radial pulse) and clinical laboratory tests, such as routine blood analysis, urine tests and medical history investigation. Pregnancy tests were done for females at screening, at admission in the Clinical Unit and at the end of the study. Only the healthy subjects who met the inclusion and exclusion criteria and had clinically normal laboratory profiles were accepted in the study.

### Study design

The study was designed as a single-dose, crossover, balanced, open label, randomized study with two treatments, two sequences and two periods. In order to prevent the carry-over effect during the two periods of the study, the wash-out time was 7 days calculated based on the applicable regulatory requirements of the FDA Guidance for Industry (5 times the elimination half-life of the given drug) as the elimination half-life ($t_{1/2}$) of dapagliflozin is known to be 12.9 hours [12].

### Study drugs

The test product (T) Dapagliflozin tablets 10 mg was developed by Sun Pharmaceutical Industries, India, while the reference product (R) used was Farxiga® immediate release tablets 10 mg manufactured by AstraZeneca Pharmaceuticals LP, Mount Vernon Ireland, for AstraZeneca Pharmaceuticals LP, USA.

### Study protocol

A randomization schedule was generated using the SAS® system for Windows release 9.1.3, SAS Institute Inc., USA, in order to randomly assign the subjects to a specific treatment during the two periods of the study. Therefore,

they alternatively received the test product or the reference product during each period of the study.

In both study periods, the IMPs were administered with 240 mL of 20 % glucose solution. In addition, approximately 60 mL of 20% glucose solution was administered every 15 minutes for up to 4 hours after the dose, as indicated by the draft guidance on generic drug development for Dapagliflozin tablets developed by the US-FDA [15]. This was considered for the safety of subjects and to prevent hypoglycaemia due to the mechanism of action of dapagliflozin.

During housing, all meal plans were identical in both periods as to ensure the same conditions for all the subjects and to reduce bias. The water intake was standardized before and after dosing of the IMP in order to reduce the variability in the IMP absorption, hence subjects were allowed to drink water as desired, except for 1 hour before and after drug administration.

The duration of each study period was of approximately 58 hours (from admission, with 10 hours before dosing, until the last sample collection, at 48 hours post dose). The required fasting period before administration of the IMP in each period was ensured by admitting the subjects with 10 hours before dosing in the Clinical Unit of Terapia SA. After admission, no food was allowed until 4 hours after dosing.

#### Blood sample collection and processing

The sampling schedule for pharmacokinetic analysis was established considering a higher sampling frequency around $t_{max}$ as predicted, in order to generate a more realistic estimation of maximum exposure. The sampling times were the following: predose and at 0.167, 0.33, 0.5, 0.66, 0.83, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 30, 36 and 48 hours post-dose in each study period. Blood samples were collected through indwelling cannula or through vein puncture from the forearm vein in vacutainers containing $K_3$EDTA as anticoagulant. The total volume of blood collected from each subject during the study did not exceed the legal limit for blood donation.

The collection and processing of the samples for the study were done under low light conditions. The collected samples were kept on ice-cold water bath until they were centrifuged under refrigeration for 15 minutes at a speed of 4000 rpm. The temperature of the centrifuge was set at 4ºC and was maintained up to maximum 10°C.

#### Bioanalytical Analysis

The separated plasma samples were further kept on ice-cold water bath until storage. Dapagliflozin and Dapagliflozin D5 used as internal standard were detected by LC-MS/MS method. The analytical method was validated for precision and accuracy and the following results were obtained: between-run precision was 0.65% to 2.49%, between-run accuracy was 88.79% to 95.10%, within-run precision was 1.12% to 5.64% and within-run accuracy was 89.44% to 94.39%. For the determination of dapagliflozin in plasma it was used a mass spectrometry method in the negative-ion multiple reaction–monitoring mode with m/z transitions of 407.10→329.20. For the internal standard Dapagliflozin D5 the obtained transition was 412.10→334.10.

Sample preparation was performed by solid phase extraction technique. The processed samples were analyzed on Gemini-NX CIS 110A, 3flm, 50x3 mm column using methanol: acetonitrile: water: ammonia solution 25 % (70:10:20:0.1 v/v/v/v) as mobile phase.

The column oven temperature was 40.00ºC ± 1.00ºC and the autosampler temperature was 10.00ºC ± 1.00ºC. The injection volume consisted of 10.00 µL and the run time was 3 minutes.

The retention time range for Dapagliflozin and Dapagliflozin D5 was 0.7 to 1.7 minutes.

For determination of dapagliflozin's peak area was used Analyst software version 1.6.3 and the concentrations of subjects' samples were calculated from the ratios of the peak area.

The calibration curves were found to be linear over plasma dapagliflozin concentration ranges of 1.01 to 352.50 ng/mL.

#### Pharmacokinetic and Statistical Analysis

For the non-compartmental pharmacokinetic analysis of dapagliflozin was used Phoenix® WinNonlin 6.3 and the calculated parametres were: $C_{max}$, $t_{max}$, observed area under the curve ($AUC_{0-t}$), total area under the curve ($AUC_{0-\infty}$), extrapolated area under the curve ($AUC_{\%\_extrap}$), and half-life time of dapagliflozin ($t_{1/2}$).

The statistical analysis was performed using SAS software version 9.1.3 for the log-transformed PK parameters ($C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$).

To determine the bioequivalence of the two products, the pharmacokinetic parameters were analyzed using ANOVA for type III square of means. The 90% confidence interval for the ratio of the test and reference product averages (least squares means) were calculated. The log-transformed pharmacokinetic parameters ($AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$) were analyzed. From analysis of log-transformed data, the ratio (T/R) of test and reference product averages for parameters $C_{max}$ and $AUC_{0-t}$ with the 90% confidence interval must be between 80% and 125% for bioequivalence.

#### Safety evaluation

Even though the safety of the subjects was not defined as an objective of this study, the subjects were monitored for adverse events. Safety measurements were carried out during the study and subjects were specifically asked about any adverse reaction after admission in both periods, before each administration of the investigational medicinal products, after dosing and during housing in the Clinical Unit, and at the end of study. From admission until the

last sampling point, a physician was always available in the Clinical Unit.

## Results and Discussion

Forty-eight healthy adult Caucasian subjects who met the inclusion and exclusion criteria described in the study protocol were enrolled in the clinical study, out of which 38 completed the study. The admitted subjects were adults, with the ages between 18-45 years, non-vegetarian diet and with a body mass index (BMI) in the range of 18.5 kg/m$^2$ to 29.0 kg/m$^2$. The demographic profile of the subjects who completed the study is shown in Table I. From the 38 subjects who finished the study, 12 were females and 26 were males. Their mean age was 29.9 (in the range of 18 – 44 and a SD of ± 7.61) and had a mean weight of 71.83 kg in the range of 50.0 – 100.0 kg (SD ± 12.332). The mean height of the subjects was 173.14 cm (SD± 12.332).

The investigational medicinal products were well tolerated by the study subjects after a single dose administrated under fasting conditions. During the study, the health status of subjects was not threatened and the recorded adverse effects did not cause study withdrawal. At the end of study safety assessment, 13 not serious adverse events were reported (see Table II). The adverse events reported were increased triglycerides, leukocyturia, increased AST, increased total bilirubin, positive nitrites in urine, decreased platelets, and leucocytosis. These were consistent with the adverse events declared in the scientific literature for dapagliflozin. Regarding vital signs, no clinically significant fluctuations were observed in the blood pressure or radial pulse of the study subjects. In addition, no clinically significant fluctuations in the blood glucose levels measurement were recorded in any of the subjects.

Following oral administration of dapagliflozin, the $C_{max}$ was attained within 2 hours under fasting state. For test formulation the mean value for $C_{max}$ was 85.47 (ng/mL), while for the reference product was 81.65 (ng/mL). The concentration versus time profiles of dapagliflozin (10 mg, single-dose) were almost identical for test and reference product after administration to healthy human subjects under fasting conditions (see Figure 1).

The mean values calculated for the pharmacokinetic parameters were similar for the IMPs (see Table III). However, for the reference product inconsiderably lower values were obtained for $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ in comparison with the test product. For test formulation, the mean $AUC_{0-t}$ was 495.13 (hr*ng/mL) and mean $AUC_{0-\infty}$ was 498.92 (hr*ng/mL), while for the reference

**Table I. Demographic characteristics of the subjects who completed the study**

| Characteristic | | Value |
|---|---|---|
| Number of subjects | | 38 |
| Gender (number) | - Women | 12 |
| | -Men | 26 |
| Age (years, mean ± SD*) | | 29.9 ± 7.61 |
| Weight (kg, mean ± SD*) | | 71.83 ± 12.332 |
| Height (cm, mean ± SD*) | | 173.14 ± 12.332 |
| Smoker | - Yes | 16 |
| | - No | 22 |

*SD – standard deviation

**Table II. Adverse events recorded during and at the study of the bioequivalence clinical trial**

| During the study | At the end of the study |
|---|---|
| 6 cases of increased triglycerides;<br>1 case of leukocyturia;<br>1 case of increased AST<br>1 case of increased total bilirubin; | 1 case of positive nitrites in urine;<br>1 case of decreased platelets;<br>1 case of atrio-ventricular block 1st degree;<br>1 case of leukocytosis |

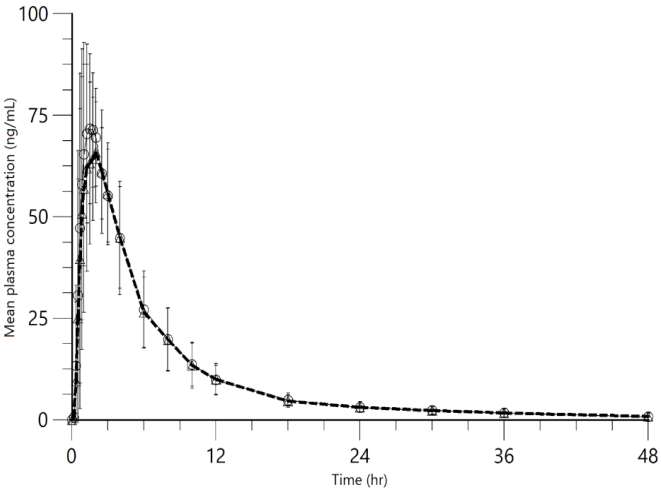


**Fig. 1. Mean plasma concentration of dapagliflozin after administration of test product Dapagliflozin 10 mg immediate release tablets, developed by Sun Pharma, India (with full line) and the reference product Farxiga®, AstraZeneca, immediate release tablets (with dotted line), in 38 Caucasian subjects under fasting conditions**

**Table III. Main pharmacokinetic parameters of dapagliflozin in 38 Caucasian healthy subjects following administration of dapagliflozin 10 mg immediate release tablets under fasting conditions**

| Pharmacokinetic parameter | Mean | | SD[1] | | CV%[2] | | Median | |
|---|---|---|---|---|---|---|---|---|
| | R | T | R | T | R | T | R | T |
| $C_{max}$ (ng/mL) | 81.65 | 85.47 | 26.32 | 25.57 | 32.24 | 29.92 | 77.32 | 76.97 |
| $AUC_{0-t}$ (hr*ng/mL) | 480.92 | 495.13 | 122.50 | 123.50 | 25.47 | 24.94 | 449.60 | 468.44 |
| $AUC_{0-\infty}$ (hr*ng/mL) | 484.02 | 498.92 | 120.49 | 121.24 | 24.89 | 24.30 | 449.90 | 472.70 |
| $t_{1/2}$ (hr) | 16.60 | 14.04 | 10.03 | 5.64 | 60.45 | 40.19 | 14.79 | 14.14 |
| $t_{max}$ (hr) | 1.80 | 1.64 | 0.71 | 0.73 | 39.45 | 44.65 | 1.75 | 1.50 |
| $k_{el}$ ($hr^{-1}$) | 0.05 | 0.06 | 0.02 | 0.03 | 46.72 | 40.19 | 0.05 | 0.05 |

SD1 – standard deviation; CV%2 – coefficient of variation; T represents Test Product (dapagliflozin 10 mg immediate release tablets developed by Sun Pharma, India); R represents Reference Product (Farxiga® 10 mg immediate release tablets, AstraZeneca)

**Table IV. Bioequivalence evaluation of pharmacokinetic parameters of dapagliflozin after administration of 10 mg immediate release tablet (test and reference), in 38 healthy Caucasian subjects, under fasting conditions**

| Dependent | Units | CI 90 Lower | CI 90 Upper | Ratio %Ref | Bioequivalence conclusion |
|---|---|---|---|---|---|
| Ln($C_{max}$) | ng/mL | 96.08 | 115.13 | 105.17 | Bioequivalent |
| Ln($AUC_{0-t}$) | hr*ng/mL | 101.02 | 105.31 | 103.14 | Bioequivalent |
| Ln($AUC_{0-\infty}$) | hr*ng/mL | 101.19 | 105.40 | 103.27 | Bioequivalent |

*CI – confidence interval (90%); Bioequivalent if 90% CI: 80.00-125.00%

product the mean results were slightly lower for both $AUC_{0-t}$ (480.92 hr*ng/mL) and $AUC_{0-\infty}$ (484.02 hr*ng/mL), respectively.

On the other hand, for $t_{1/2}$ (hr) the mean value obtained was slightly higher for the reference product (16.60 hr) than for the test product (14.04 hr). Regarding the mean $t_{max}$, the value obtained for test product was 1.64 hours while for reference was 1.80 hours.

The results of the bioequivalence assessment under fasting state of subjects and the conclusion of bioequivalence for the evaluated PK parameters are summarized in Table IV.

The 90% confidence intervals for the ratio of test (T) and reference (R) product averages (least squares means) derived from the analysis of log (natural) transformed pharmacokinetic parameters $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were within 80.00-125.00% acceptance range. For $C_{max}$ the ratios of least-squares means (with 90% confidence intervals) was 105.17% (96.08% – 115.13%). For $AUC_{0-t}$ and $AUC_{0-\infty}$ the ratios of least-squares means (with 90% confidence intervals) were 103.14% (101.02% – 105.31%), 103.27% (101.19% - 105.40%), respectively.

## Conclusions

Taking into consideration these results, the immediate release tablets newly developed by Sun Pharmaceutical Industries, India, containing Dapagliflozin 10 mg, were concluded to be bioequivalent with the reference product (Farxiga®, AstraZeneca Pharmaceuticals LP, USA) in healthy adult Caucasian subjects under fasting conditions.

## Authors' contribution

MO (Data curation; Formal analysis; Writing – original draft)

AM (Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing – review & editing)

AM Gheldiu (Data curation; Formal analysis; Software; Writing – review & editing)

DIP (Data curation; Formal analysis; Software)

SB (Funding acquisition; Investigation; Project administration; Resources; Supervision)

AK (Funding acquisition; Investigation; Methodology; Supervision; Validation)

LV (Formal analysis; Investigation; Supervision; Validation; Visualization; Writing – review & editing)

## Conflict of interest

None to declare.

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LETTER TO EDITOR

# The First International Western and Chinese & Thai Medicine Workshop on Pain Therapy & Management, Targu Mures, Romania. November 25 – 28, 2019

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The First International Workshop on using combined Western and Traditional Medicine for Pain Therapy was successfully held in Romania, 2018. Medical doctors from Romania, China and Thailand participated as lecturers and demonstrators to more than 40 participants. At the conclusion of the workshop, the organizers, lecturers and participants overwhelmingly endorsed the proposals to organize the second workshop and to organize a clinical trial in developing a combined protocol for innovative pain therapy.

**Keywords**: western and traditional medicine, pain therapy, acupuncture, therapeutic massage, osteoarthritis

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## Report

The 4-day workshop was successfully organized and executed at the Advanced Research Center of the University of Medicine, Pharmacy, Science and Technology (UMFST). The workshop was co-sponsored by the University of Medicine, Pharmacy, Science and Technology, Romania (UMFST), Shantou University Medical College (SUMC), China and Thammasat University (TU), Thailand. More than 40 participants registered for the workshop. They included doctors from UMFST, Targu Mures and other cities in Romania, as well as staff and medical students. In addition, six Thai Traditional Medicine students came from Thailand to attend the workshop. The lecturers included 2 traditional Chinese medicine doctors from China (Yong Xiao, Jiasheng Chen), 3 traditional Thai medicine doctors from Thailand (Pannawat Chaiyawatthanananthn, Puritat Kanokkangsadal and Arounporn Itharat), 1 Romanian doctor who specialized in homeopathic and ozone therapies (Adrian Alecu), and 2 Western medicine doctors from UMFST (Horatiu Popoviciu and Monica Copotoiu). A variety of activities from the Workshop are shown in Figure 1.

The workshop started with a short opening ceremony. Although Rector Azamfirei was unable to attend, three Vice Rectors (Borda, Marginian and Enachescu) came to show their endorsement of the workshop, and to represent the Rector. The workshop was organized, in general, to have interactive lectures during the mornings and demonstrations with volunteers during the afternoons. Therefore, both lecturers and participants were active in communicating with each other for 4 days. After their sharing of knowledge and technologies from the three countries, the afternoon of the 4th day was used to discuss how to organize collaborative projects. With active communication and support from everyone, the most important agreement was the organization of a tri-national project for the three participating countries. The project will be an unprecedented clinical trial involving Western medication, Chinese acupuncture and Thai massage on treatment of specific types of patients who have difficult-to-cure chronic pain. This is anticipated to have a 3-month therapeutic phase with a 3-month follow-up phase. The protocol will be harmonized among the three countries together with specific monitoring and evaluation procedures. The overall goal of this international collaborative project is to develop highly innovative therapeutic protocols which can bring significant improvement of medical care to patients around the world.

Besides having excellent academic value, the workshop also provided ample of opportunity to share cultural experience. For example, the 6 Thai students performed Traditional Thai dancing which was complimented by Traditional Romanian dancing during the Farewell Dinner.

The clinical trial and other agreed collaborations will form the foundation for the proposed Center of Traditional and Western Medicine for Innovative Therapy at UMFST. In addition, several participants have requested and volunteered to organize the second international workshop in UMFST within two years.

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**Fig. 1. Top row: Delegations from China and Thailand with two of the organizers (Au and Alecu) were interviewed on TV by the university; Second row: Dr. Xiao demonstrated acupuncture and Dr. Chen demonstrated cupping procedures on volunteers; Third row: Dr. Puritat and Thailand students demonstrated Thai massage and exercise to reduce pain and stress; Bottom row: 6 Traditional Thai medicine students performed traditional Thai dances, together with Traditional Romanian dancers.**

## Acknowledgement

With deep appreciation to the strong support from Rector Azamfirei and the other administrators of UMFST; members of the organizating committee (Nicolae Neagu, Minodora Dobreanu, Horatiu Popoviciu, Adrain Alecu, Monica Copotoiu, Dan Szabo and Alexandra-Camelia Gliga); members of the technical support team (Siviu Morariu, Catalin Dogaru, Teodora Mandru, Kinga Bota, Lucian Morariu, Cristina Tecan and Anamaria Fodor); volunteer team (Denisa Capra, Mădălina Ciobanu, Andreea Ilieş, Daniel Sima, Theodora Ciulea, Csatlós Attila Tamás, Agnes Antal and Roxana Mihaela Munteanu), staff in the Alma Mater restaurant and hotel, and the driver team.

# Statement of ethics

The journal observes and values the principles of ethics in scientific research, as previously highlighted in the main document on the topic: "The code of ethics for scientific research of the George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures" and according to all the documents enlisted as source of our code. We promote innovative research and original articles are thus prioritized. The editors adhere to the views and recommendations of the International Committee of Medical Journal Editors.

All forms of plagiarism will be exposed when and if evidenced. Therefore, we discourage ghost writing and guest authorship, including the situation when the author was unaware of being enlisted as co-author and as such, the cover letter is invalidated.

The peer-review process is intended to be a complimentary supplemental investment of expertise and is highly valued when well accomplished. The best peer-reviewers will be acknowledged by the editors and we promise to drop out the peer-reviewers who repeatedly acted as if their task was burdensome or annoying, and came up with shallow reports.

The journal encourages PhD students to publish their research. Reviews and "state of the art" articles will be hosted upon invitation. When judging the quality of the research, double standards are not an option.

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Errata will be inserted as soon as an error is detected, either spontaneously or by authorized notice.

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