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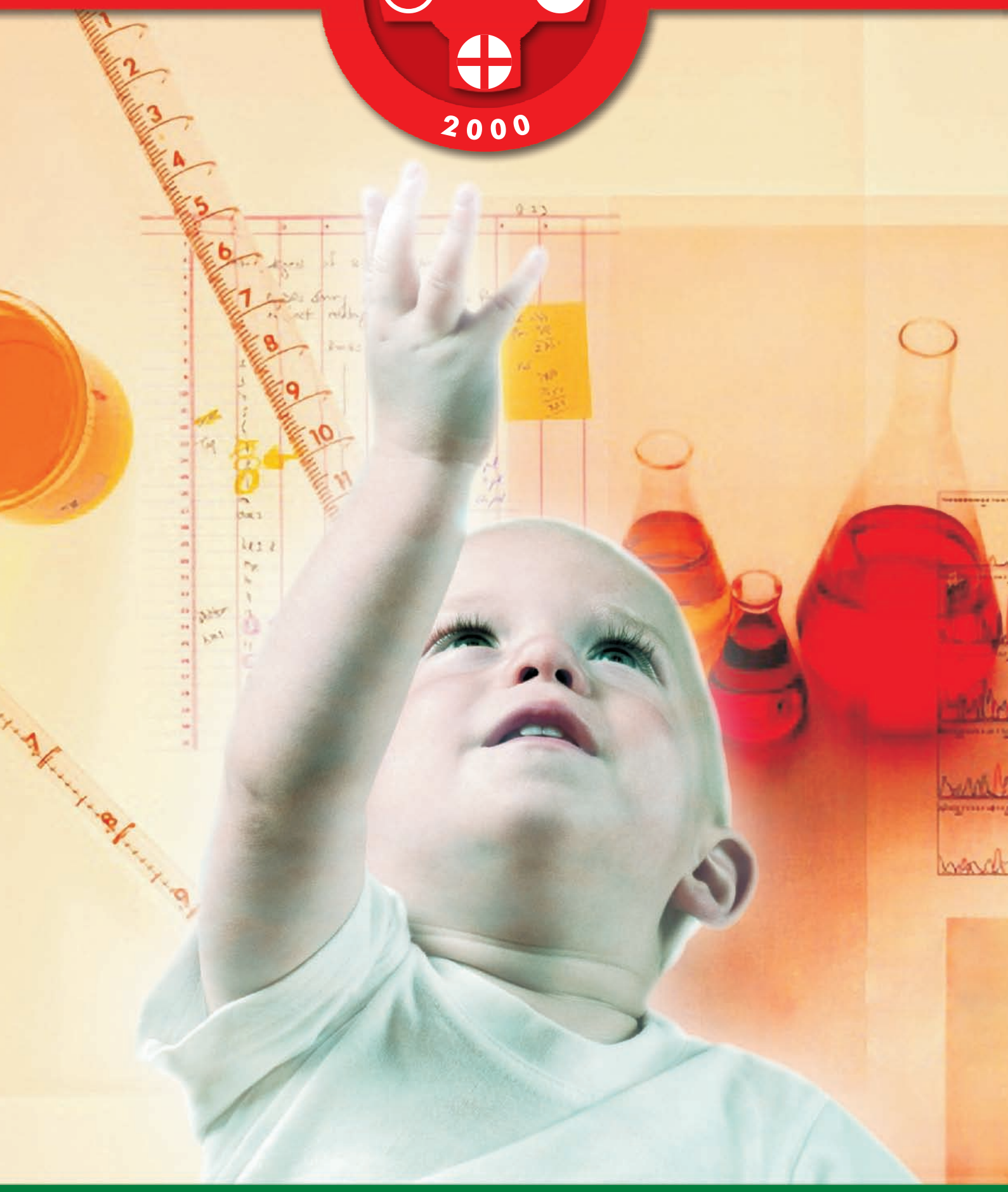

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Table Of Contents

GENERATION OF SOMATIC CELLS BY DIRECT CONVERSION – DO WE NEED PLURIPOTENT CELLS?

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ABSTRACT

The pluripotency of embryonic stem cells (ESCs) makes them a potentially attractive resource for generating clinically useful somatic cells except for the problem of immune rejection. In effect, a transplant of cells differentiated from an ESC line is no different than receiving cells from the individual that would have developed from the embryo that from which the ESCs were originally derivedfrom. In view of this, it should not be surprising that the recipient's immune system could attempt to reject the incoming "foreign" cells. This is the primary reason why techniques to make individualised human pluripotent stem cells hhave been intensively investigated over the last twenty years. Initial attempts focused on the possibility of "therapeutic cloning", the deliberate creation of a human embryo by transfer of a somatic nucleus from the intended recipient into an oocyte from a human donor, with the aim of creating a tailor-made stem cell line from that embryo's inner cell mass;, however, this method has not yet been successful (1,2). Therapeutic cloning may indeed have been rendered obsolete by the technique of induced pluripotency (3,4), and if the products of this method, namely induced pluripotent stem cells or iPSCs, are truly equivalent to ESCs, this could be the way to usher in the long promised age of personalised regenerative medicine. There are still substantial problems to overcome before this becomes reality. Even if iPSCs and ESCs are equivalent, we still need to develop reproducible methods to direct their differentiation into clinically useful cells or tissues in a cost-effective manner. Moreover, we must ensure that the resulting cells are functionally equivalent to their adult body counterparts and do not create additional health problems years after their administration. In short, there is still much work to be done.

EARLY ATTEMPTS AT CELLULAR ALCHEMY

There may be other ways to produce clinically useful differentiated cells that do not require the formation of pluripotent stem cells. This might be advantageous because pluripotency seems to compromise the cells ability to give rise to equivalent adult somatic cells. Once a genome has been reverted back to an embryonic state, it is difficult to obtain somatic cell types that differentiate from this state to function as though they were part of the adult body. Thus, how could we make the desired cell types in other ways? One possibility would be to convert one adult somatic cell type into another by reprogramming its gene expression profile (or transcriptome). Cell fusion studies suggested that the cytoplasm of ESCs was able to reprogram fibroblast nuclei into a pluripotent state (5), which supports the idea that the transcriptome of cells may be altered more readily than previously imagined. In addition, other data suggested that some somatic cells were able to change into other types under the appropriate culture conditions, which begs the question "can we convert one cell type to another simply by exposing the target cell to the cytoplasm of the other cell type?" In 2002, studies published by the laboratory of Phillipe Collas suggested that exposure of human fibroblasts and fibroblast nuclei to extracts from T cells changed the transcriptome of the fibroblasts to resemble that of the T-cells (6). The technique was further developed by the transient permeabilisation of whole cells using streptolysin O followed by exposure to a T-cell extract treated with RNaseI to eliminate the possibility of fibroblast transcription of transferred T-cell mRNA. Fibroblasts exposed to the extract were resealed by treatment with dilute calcium chloride and then expanded in culture. Reprogrammed cells began to express surface antigens typical of haematopoietic cells 24-60 hours after exposure to the T-cell extract, indicating that these were newly translated molecules and had not been simply carried over in the extract. Furthermore, acetylation of the promoters of the genes encoding such proteins suggested that epigenetic reprogramming of the fibroblast genome had taken place to impose the transcriptome of the T-cell, at least in part on a different genome. The reprogramming ability was not confined to T-cell extracts, as Collas' group went on to demonstrate that the treatment of primary rat fibroblasts with extracts from a rat insulinoma cell line induced expression of the pancreas-specific *Pdx1* and insulin genes (7). This was followed by directed differentiation of pluripotent mouse ESCs into type II pneumocytes (8) and cardiomyocytes (7); however, expression of the genes upregulated by reprogramming was often tran-

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sient depending on the nature of the type of cell derived. Rat insuloma reprogrammed fibroblasts could only maintain Pdx1 and insulin gene expression for several days, although Jurkat cell extract treated 293T cells were able to maintain the new transcriptome over three months, albeit with progressive decreases in expression levels (9). This implies that the epigenetic reprogramming requirement for exogenous gene upregulation is not permanent. Conversely, there is evidence from studies from Collas' group and others that the newly imposed epigenetic modifications are heritable because activating histone modifications are detectable more than a week after extract exposure in cells that are known to divide every 24 hourshrs . Not all possible histone modifications that could constitute a stable gene expression control system were measured in these cells, and, in any case, these are more labile and subject to frequent change than the overall levels of DNA methylation at the promoters of upregulated genes. Bisulphite sequencing allows us to identify methylated cytosines in CpG dinucleotides (10), which has shown substantial demethylation of the *OCT4* promoter in mouse fibroblasts treated with mouse ESC extracts. Again, the stability of this DNA methylation is uncertain and has only been analysed in detail for somatic cells reprogrammed by ESC extracts in an attempt to generate pluripotent stem cells. It is possible that reprogramming using somatic cell extracts (such as cardiomyocytes) may not lead to such extensive demethylation of target genes, which could account for the instability of the imposed transcriptome.

IMPROVING DIRECT INTERCONVERSION OF SOMATIC CELLS

The reprogramming phenomenon shown in previous studies likely resulted from the presence of cytoplasmic "factors" in the cells used to make the reprogramming extract that were not identified by Collas' or other groups. Identifying and applying these factors might generate an improved strategy for generating specific cell types as long as the factors are specific to the desired cells and capable of reprogramming. Several studies have now indicated that ectopic overexpression of isolated factors can turn one differentiated cell type into another, although this is usually within the same lineage. This means that cells derived from the mesoderm during embryogenesis tend to be able to produce only other types of mesodermal cells. The same seems to hold true for cells of the ectoderm and endoderm. Thus for the most part, these differentiations are not considered to undergo transdifferentiation.

DIRECT CONVERSION TO NEURONS

The first example of this type of conversion was the conversion of mouse and human fibroblasts into functional neurons by transfection of the former with only three neuron-specific transcription factors. Following the argument that the transcription factors that most closely defined the neuronal transcriptome, a total of 19 neuron specific genes were packaged into lentiviral vectors and used to transfect mouse tail tip fibroblasts. Those combinations of transcription factors required to induce neurogenesis were narrowed down to *Ascl1, Brn2* and *Myt1l* (11). The fibroblasts were obtained from 3-day-old *Tau-GFP* and Rosa26-*rtTA* mice to enable the detection of neural progenitors via their expression of green fluorescent protein (GFP) under the control of the *Tau* promoter (a neural-specific gene). GFP expression was detectable a few days after transfection, and after FACS enrichment, these cells were able to integrate into the layers of previously cultured neonatal cortical neurons. Thus, the ability of the direct conversion cells to form functional synapses was demonstrated. Electrophysiological characteristics of the cultured Tau-GFP cells were measured by patch clamp, and they were shown to generate repetitive action potentials similar to those of mature neurons.

CARDIOMYOCYTES FROM FIBROBLASTS

The group led by Sheng Ding at the Scripps Research Institute is well known for its publications describing the influence of small molecules on the epigenetic reprogramming process of iPSC derivation, but in December 2010, they published a method for the direct conversion of mouse embryonic fibroblasts (MEFs) into differentiated cardiomyocytes. Although, in this case, they used a similar method to iPSC induction but with culture conditions designed to favour cardiogenesis. As for the neuronal conversions above, they made use of a myocardium-specific reporter gene driving LacZ expression, but transfection of the MEFs was carried out with *Oct4, Sox2* and *Klf4,* which would normally be expected to drive the fibroblast genome towards pluripotency when exposed to the appropriate culture conditions. The presence of cardiogenic cells was indicated by transient, widespread β-galactosidase expression, and modification of the culture medium to a progressively lower concentration of fetal bovine serum and inhibitors of JAK-STAT signalling enhanced the number of cells presenting mid-stage cardiac markers, such as *Flk1, Nkx2.5* and *Gata4,* approximately 9 days after transfection. Late-stage cardiac markers such as cardiac troponin appeared after 11 days, and areas of spontaneously contracting tissue appeared on day 15. Addition of the cardio-inductive growth factor Bone Morphogenetic Protein 4 (BMP4) increased the number of contracting areas by nearly 150-fold (12). The contraction rate was variable, ranging from 4-130 beats per minute, but, they also exhibited calcium transient and electrophysiological action potentials similar to those reported by other groups, which suggested a primarily atrial phenotype for the cardiomyocytes generated in this work.

The method used to generate cardiomyocytes in this manner is interesting because it suggests that the fibroblasts may have been reprogrammed to a pluripotent state

before re-differentiating down a cardiogenic pathway. Although this cannot be formally ruled out, it is an unlikely scenario because commitment to the cardiac lineage occurs soon after transfection with the lentiviral vectors, and typical indicators of the onset of a pluripotent state, such as the upregulation of the endogenous copy of *Nanog*, are scarcely observable. Examination of the latter point using fibroblasts obtained from mice carrying a *Nanog*-GFP reporter construct suggested that pluripotent cells were not present at the time points from which cardiac markers appeared in the cell cultures. Bonafide markers of pluripotency only appeared under growth conditions that promoted formation of iPSCs and at much later time points after transfection. Taken together these observations point to the emergence of cardiac lineage cells from non-pluripotent precursors.

MAKING BLOOD

A key observation made by the Bhatia group was the expression of the pan-haematopoietic marker CD45 in some colonies of cells during iPSC derivation. Approximately ten days after transfection with *OCT4, SOX2, KLF4* and / or *c-MYC*, the cells no longer resembled the parent fibroblasts but did not have any similarity to genuine iPSCs. The presumption is that these are cells at some intermediate stage of dedifferentiation, but it is not yet clear if reprogramming entails progressive reversal of lineage commitment or erasure of the epigenetic information controlling cell identity over a short timeframe. Bhatia's suggestion is that the bulk of the epigenetic erasure is performed by the ectopic expression of *OCT4,* but this factor cannot induce pluripotency on its own and needs SOX2 and KLF4, at least, to complete the process. No markers of pluripotency such as Tra-1-60, which can normally be detected by day 21 of normal iPSC induction, were observed, and *OCT4* transduced fibroblasts were unable to generate teratomas in immunodeficient mice (13). *OCT4* overexpression produces detectable numbers of CD45 expressing cells 21 days after transfection, and although this method is a departure from the transcription factor screening technique employed to select neurogenic factors, it presents a tantalising possibility of making blood cells for autologous transplant into adults.

Gene expression analysis of the OCT4-CD45-expressing cells (enriched by FACS for the CD45 cell surface antigen) showed a high degree of similarity to haematopoietic progenitors derived from peripheral blood or umbilical cord blood, which implies a possible haematopoietic function for these cells. Expansion of OCT4-CD45 cells in the presence of cytokines that support haematopoietic progenitor development gave rise to progeny expressing surface antigens characteristics of a range of differentiated haematopoietic cell types such as monocytes (CD14) and myeloid progenitors (CD33 and CD13) in addition to cells with typical neutrophil, eosinophil and basophil morphologies. OCT4-CD45 cells transplanted into myeloablated immunodeficient NOD/SCID IL2Rγc-null mice contributed largely to the development of cells with a myeloid phenotype, although the level of engraftment was comparable to that obtained with umbilical cord blood progenitors. Importantly, the primary engrafted OCT4-CD45 cells showed only limited ability to engraft a secondary NOD/SCID IL2Rγc-null mouse suggesting that their ability to undergo indefinite expansion is reduced compared with haematopoietic progenitors derived from pluripotent cells. This implies that the OCT4-CD45 cells may be a safer alternative in view of the tendency of pluripotent derived haematopoietic cells to undergo leukemic transformation. Erythropoiesis from the OCT4-CD45 cells provided an interesting contrast to pluripotent stem cells in that treatment with erythropoietin (a growth factor that induces- erythropoiesis *in vitro* and *in vivo*) resulted in the production of CD71 expressing erythroblasts expressing glycophorin A and the adult β-globin protein. Despite this, the OCT4-CD45 cells were only able to complete the differentiation of the megakaryocyte-platelet lineage, and erythrocytes were not detected. Moreover, lymphopoiesis was not observed despite expression of CD34 in 25% of the OCT4-CD45 cells, which indicated the possible presence of more primitive haematopoietic progenitor cells that were capable of multilineage differentiation. Apart from the expression of CD45 following OCT4 transfection, the development of the subsequent haematopoietic cell types seemed to depend on the presence of haematopoiesis promoting cytokines. Therefore, it is possible that the cell types detected in this study arose from a haematopoietic progenitor or more committed blood cell type present in the dermal fibroblast samples.

HEPATOCYTES

In a similar approach to the screen for inducers of neurogenesis, combinations of the transcription factors *Hnf4α, Foxa1, Foxa2* and *Foxa3* were shown to convert mouse embryonic stem cells and adult fibroblasts into hepatocytes. Two weeks after transfection with individual pools of two factors, the cells were replated onto collagen. Three weeks after the replating step, clusters of cells with an epithelial morphology and normal karyotype appeared and could be maintained in culture for several passages (referred to as iHep cells) (14). These did not express markers typical of fibroblasts, but they were strongly positive for E-cadherin, albumin and the canalicular membrane protein multidrug resistance protein, Mrp2,; all of which are typical of hepatocytes. iHep cells had a similar transcriptomic profile to *ex vivo*-derived hepatocytes and showed several functional similarities such as production of urea, synthesis of triglycerides and cytochrome P450 activity. In contrast to the haematopoietic data generated by Bhatia's group, iHep cells had a greater similarity to terminally differentiated hepato-

cytes because there was little evidence of more primitive endodermal progenitors (such as pancreatic or intestinal cells) after transfection with any of the transcription factor combinations. There was also little convincing evidence of bi-potent hepatic progenitor cells (cholangiocytes) due to lack of cytokeratin 7 expression. Regardless, iHep cells appeared to function *in vivo*. Hepatocytes isolated from the adult mouse liver are able to reconstitute the hepatic tissues of mice deficient for the enzyme fumaryl acetoacetate hydrolase (FAH), which are used to model liver injury (15). One month after intrasplenic injection of iHep cells into FAH-/- mice, FAH-expressing hepatocytes had engrafted and reconstituted the hepatic tissues resulting in rescue from liver failure. Conversely, all the FAH-/- mice transplanted with the untransfected fibroblasts (i.e., non-iHep cells) died within 27 days.

As with the other examples of direct conversion described in this review, there is still the possibility that iHep cells could have been derived from endodermal progenitor cells present in the fibroblast population used for transfection. This was particularly true for the earliest experiments of this research group, as they made use of MEFs obtained by dissection of E12.5 mouse embryos. Because it is not easy to completely remove the embryonic liver and other components of the digestive system from such embryos, it is thus possible that endodermal progenitor cells were present in the MEF culture; however, iHep cells were also obtained from adult mouse dermal fibroblasts. As mesenchymal stem cells (MSCs) have been shown to transdifferentiate into hepatocyte-like cells (16), another consideration is the possible presence of MSCs in the fibroblast cultures. However, against this possibility are the observations that iHep cells arise with similar frequency from both enriched MSCs and fibroblast cultures.

MECHANISMS OF DIRECT CONVERSION FROM FIBROBLASTS

From the limited examples of direct conversion in the scientific literature, it is difficult to hypothesise about possible mechanisms that may reprogram one cell type into another. Two of the above examples involve transfection of at least one transcription factor associated with the pluripotent state, and although the authors of these publications have gone to considerable lengths to rule out the reprogramming of the fibroblast genome back to a pluripotent state followed by re-differentiation to the target cell type, it is still possible that pluripotency transcription factor may contribute to direct conversion. The capacity of Oct4 for epigenetic reprogramming has been well documented, and iPSCs have been generated from neural stem cells (NSCs) from the adult subventricular zone by transfection of Oct4 alone (17, 18), implying that the Oct4 gene product is able to select some (or perhaps all) of its binding sites within the NSC genome. In addition, iPSCs can be generated by a combination of Oct4 transfection and treatment with small molecules that inhibit certain chromatin-modifying enzymes (19). In view of these data, Oct4 could be a master regulatory gene of the pluripotent phenotype, but it requires other gene products to induce true pluripotency. SinceAs zygotic deletion of Oct4 causes mouse epiblast cells to undergo extensive chromatin compaction, it is possible that one of the functions of Oct4 is maintenance of an "open" chromatin conformation (20). This implies (and other groups have speculated) that Oct4 may perform this task by ensuring that the epigenetic modifications associated with chromatin compaction are not imposed upon the pluripotent genome. The converse of this argument may also be that it ensures the removal of such modifications from a somatic genome and imposes a much greater degree of differentiation plasticity upon the cell. This is not the same pluripotency as we find in ESCs, but it may allow more access to transcription factors that favour differentiation down several lineages and expression of a wider range of transcription factors associated with varied cell identities. The conditions under which we grow Oct4-expressing cells may then favour the survival of some somatic cell types over others, or they may induce signal transduction mechanisms that instruct lineage-specific differentiation. Both of these possibilities have been invoked to explain the direct conversion of fibroblasts to cardiomyocytes and haematopoietic cells, indicated in the publications described earlier.

It is more difficult to explain why ectopic expression of Hnf4α with one of more of the Foxa genes is sufficient to convert fibroblasts into iHep cells; however, potential clues may lie in a possible chromatin-modifying function of the Foxa gene family. Mammals have three seemingly unlinked *FoxA* genes (*FoxA1*, *FoxA2*, and *FoxA3*), of which *FoxA2* has functions closely linked to endoderm development (21, 22). Ectopic expression of *FoxA2* promotes endoderm development in ESCs (23), and it has also been shown that *FoxA* genes, as part of the more general class of Fox or forkhead box genes, function in diverse developmental and signal transduction mechanisms (24). A major aspect of this function seems to be maintenance of an open chromatin conformation that is reminiscent of the similar, albeit probably more extensive, function of Oct4. The purpose of this appears to be pre-conditioning of the genomes of endoderm progenitor cells to activate liver, pancreas or other tissue-specific genes, and for this reason, *FoxA* genes have been termed "pioneer" factors. In view of this, it is possible that ectopic expression of one or more of the *FoxA* genes needed to induce iHep cell formation may be the determinant of epigenetic reprogramming of the fibroblast genome.

Another possibility is that fibroblasts have an inherent plasticity that allows them to differentiate more readily. Although not extensive, there is evidence in the literature suggesting that human dermal fibroblasts can

differentiate into cells representative of all three embryonic germ layers when cultured under appropriate conditions (25). The cell types reported in this recent publication include neuronal (indicated by nestin and β-III-tubulin expression), immature myoblast-like cells (Myf5 and Desmin expression) and possible insulin-synthesising cells that are indicative of endoderm. There are additional data indicating that fibroblasts are capable of multi-lineage differentiation, and we cannot formally rule out the presence of progenitor or tissue-specific stem cells in the fibroblast culture. However, this apparent plasticity is worth bearing in mind for future studies of direct conversion.

CONCLUDING REMARKS

Induced pluripotent stem cells are relatively easy to generate and expand in culture, but we have to be certain they can produce safe, clinically useful and economically viable cell products before they can have a major impact on regenerative medicine. Therefore, it is possible that direct conversion methods may have advantages in terms of the possibilities of generating cells that have greater similarity to those found in the adult body. There are enormous hurdles to overcome: the phenomenon is restricted to a small set of very recent observation, and there are no guarantees that this concept will be universally applicable to all clinically desirable cell types. Furthermore, we have no data concerning the mutational load present in directly converted cells. If these are derived from tissues of older individuals, they could have accumulated significant levels of damaged DNA in both the nuclear and mitochondrial genomes. They may have shorter telomeres, and as some publications suggest, epigenetic changes may accumulate as a function of organismal age (26 – 29). Although iPSCs may be able to reset damage such as telomere lengths, we cannot be certain that direct conversion will do this .

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ADVERSE EVENTS INDUCED BY ANTI-INFECTIVES IN HOSPITALIZED PATIENTS

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NEŽELJENI DOGAĐAJI UZROKOVANI PRIMENOM ANTIMIKROBNE TERAPIJE KOD HOSPITALIZOVANIH PACIJENATA

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ABSTRACT

Introduction. *The objective of this study was to obtain accurate data about adverse events (AEs) related to antimicrobial therapy, including rate, causality, outcome and circumstances in which they occurred.*

Methods. *Hospitalizised patients undergoing treatment with one or more anti-infective drugs were eligible for the study. The main outcome measures were any adverse clinical signs, symptoms or laboratory test abnormalities that had likely been induced by an anti-infective agent. The seriousness and causality of the AEs were classified on the basis of WHO recommendations and compared with results from the Naranjo Probability Scale, European Causality Categories (ABO system) and French Imputation System assessments.*

Results. *During the 6-month study period, 421 patients (72.84%) received at least one anti-infective drug. Thirty-one patients (7.36%) were found to have anti-infective-induced AEs. Anti-tuberculosis agents caused the highest percentage of AEs (38.7%) during the period of observation. The majority of AEs presented as disorders of the gastrointestinal tract (41.9%).*

Conclusion. *AEs in females tend to last longer than in males. Males have a greater risk of experiencing AEs caused by fluoroquinolones (e.g., ciprofloxacin), while female patients have a greater risk of experiencing AEs caused by anti-tuberculosis agents. The incidence of AEs in inpatients receiving anti-infectives in our study (7.36%) is within the range reported from other studies. There is a great need for the development of new causality assessment scales that have better sensitivity and specificity.*

Key words: *adverse drug event, anti-infective agents, hospitalisation*

SAŽETAK

Uvod. *Cilj studije je da se utvrde precizni podaci o neželjenim događajima uzrokovanim primenom antimikrobne terapije, o stopi njihove učestalosti, uzročnosti, ishodu i okolnostima pod kojima su se pojavile.*

Metode. *Posmatrani su pacijenti koji su tokom hospitalnog lečenja bili tretirani anti-infektivnim lekovima i kod kojih je došlo do pojave neželjenog kliničkog znaka, simptoma ili abnormalnosti laboratorijskih nalaza, za koje se može postaviti sumnja da su indukovani primenom anti-infektivne terapije. Težina i uzročnost neželjenih događaja vezanih za primenu lekova (NDL) su klasifikovane na osnovu preporuka SZO i potom poređene sa rezultatima dobijenim iz analize Naranjo skale verovatnoće, Evropskih kategorija kauzalnosti (ABO sistem) i Francuskog sistema za utvrđivanje kauzalnosti.*

Rezultati. *Tokom 6-mesečnog studijskog perioda, 421 pacijent (72.84%) je dobio najmanje jedan anti-infektivni lek. Kod 31 pacijenta (7.36%) je uočen neželjeni događaj, za koji je postavljena sumnja da je uzrokovan primenom anti-infektivne terapije. Najveći procenat NDL u posmatranom periodu je bio uzrokovan antituberkuloticima (38.7%). Većina neželjenih dejstava lekova se pojavila kao poremećaji digestivnog trakta (41.9%).*

Zaključak. *Neželjena dejstva kod žena imaju tendenciju da traju duže nego kod muškaraca. Muškarci imaju veći rizik da iskuse neželjene događaje uzrokovane fluorohinolonima (ciprofloksacin) od žena, dok su žene u većem riziku da iskuse neželjene događaje uzrokovane antituberkuloticima. Incidenca NDL kod pacijenata u našoj studiji koji su primali antiinfektivnu terapiju (7.6%) je u proseku rezultata koje su dale druge studije koje su se bavile sličnim problemom. U zaključku se nameće velika potreba za razvojem novih skala za procenu kauzalnosti, sa boljom senzitivnošću i specifičnošću.*

Ključne reči: *neželjeni događaj povezan sa primenom leka, anti-infektivni agensi, hospitalizacija.*

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INTRODUCTION

Adverse drug reactions (ADRs) are very common in everyday medical practice and cause various problems for patients and physicians, not only the with regard to their management but also in their recording, classification and assessment. They are regarded not only as a medical issue but also as an economic problem asbecause they often lead to an increase in the length of hospitalisation and a need to administer additional medications. An estimation made in France in 2001 suggested that increased ADR-induced costs result especially from the prolongation of hospitalisation, with the average cost increase calculated at 4150 euros per ADR.[1] A prospective analysis of 18820 patients in Britain projected the annual National Health Service costs of hospital admissions due to ADRs of up to 706 million euros.[2]

The importance of this problem is imposing the need to conduct more studies on drug safety and assessment of various adverse events. These results may greatly influence medical practice in the future, leading to safer and more rational therapy. This would greatly benefit patients and health systems overall. The severity and substantial costs of ADRs in hospitals justify investments aimed at preventing these events.[3]

The incidence of ADRs in hospitalised patients ranges from 1.5% to 35%[4,5], depending on various factors. Anti-infective agents are responsible for approximately 25% of ADRs in hospitalised patients.[6]

However, the true incidence of ADRs in hospital departments specialised for the care of patients with disorders of a single organ system remains to be established. The aim of our study was to investigate the rates and types of adverse events related to antimicrobials among patients with pulmonary disorders.

METHODS

The study was planned as a prospective, cross-sectional study, and it was conducted at the Center for Pulmonary Diseases of the Clinic for Internal Diseases, Clinical Center Kragujevac, Serbia, over a 6-month period from December 2005 until May 2006. The Center for Pulmonary Diseases has a capacity of 60 beds, and it was assumed that approximately 600 patients would be hospitalised during this 6-month period. Only hospitalised patients receiving one or more anti-infective drugs were eligible for the study. The most common reasons for hospitalisation and administration of anti-infective therapy were the following: infections of the lower respiratory tract, lung tuberculosis and exacerbation of chronic obstructive pulmonary disease (COPD). Each patient's health status, both subjective and objective, and the results of haematology and biochemistry laboratory tests were regularly assessed to identify and record adverse events (AEs). Patients were interviewed and physically examined on a daily basis during hospitalisation. If any abnormal clinical signs, symptoms or laboratory tests were detected that had likely been induced by an anti-infective agent, the investigator completed a pre-designed form/questionnaire.

The data obtained from the adverse event (AE) causality assessment using the WHO Causality Categories[7,8] were compared with data obtained for each AE from an analysis performed based on the Naranjo Probability Scale[9], European Causality Categories (ABO system) [10] and the French Imputation System[11].

The pre-designed form/questionnaire that was used to record AEs related to anti-infectives contained general information on the patient (name, age, sex, weight, occupation) and information concerning his/her disease, length of hospitalisation, concomitant medications, a brief description of the AE, time of onset, duration, information concerning drug discontinuation and eventual re-administration, results of laboratory analyses, previous experience with adverse reactions to drugs, information on the drug and drug package and the outcome of the AE.

All verified AEs were also reported to the Serbian National Pharmacovigilance Center using a special form the.

Statistical analysis

All experimental and clinical data were processed using SPSS software for Windows 11.0. For statistical data processing, objective mathematical and statistical tests and methods were used, which were completely adapted to the type and extent of information.

We used descriptive statistics and non-parametric statistical tests (Chi-squared test, Spearmann rank-correlation) and non-parametric analysis (Kruscal-Wallis test). The results were considered significant if $p \leq 0.05$.

RESULTS

During the study period, a total of 578 patients were hospitalised. The average age of patients was 63.34±14.25 years, and the overall average length of hospitalisation was 18.16±14.36 days. The average length of hospitalisation in patients receiving anti-tuberculosis agents was 49.05±22.46 days, and the average length in patients receiving all other anti-infectives was 15.86±13.42 days. Four hundred twenty-one patients (72.84%) received at least one anti-infective drug and were therefore eligible to enter the study. AEs induced by anti-infectives were recorded in 31 patients (7.36%). All AEs caused by anti-infective drugs occurred during hospitalizisation. The average length of hospitalisation in patients who experienced AEs was 20.13±11.53 days.

Of the 31 patients who experienced AEs, 15 were males (48.39%) and 16 were females (51.61%). Of the 421 patients who received anti-infectives, 111 (26.36%)

Table I. Number of prescriptions of anti-infective agents during the study period

Anti-infective agent	No. of prescriptions
Fluoroquinolones	150
Cephalosporins	111
Macrolides	83
Aminoglycosides	78
Sulphonamides	54
Penicillin (+ β-lactamase inhibitors)	47
Anti-tuberculosis agents 4-drug therapy	36
Anti-tuberculosis agents 5-drug therapy	4
Lincosamides	11
Metronidazole	7
Carbapenems	4
Fluconasole	4
Tetracyclines	3
Glycopeptides	2

Table III. Results of the causality assessment using the European Causality Categories – ABO system

Categories	Frequency	Per cent
A	18	58
B	13	42
O	0	0

A The reports include good reasons and sufficient documentation to assume a plausible, conceivable, likely, but not necessarily highly probable, causal relationship .

B The reports contain sufficient information to accept the possibility of a causal relationship (not impossible and not unlikely), although the connection is uncertain and may be even doubtful, e.g., because of missing data, insufficient evidence or the possibility of another explanation.

O In the reports, causality is, for one reason or another, not assessable, e.g., because of missing or conflicting data.

Table IV. Results of the causality assessment using the Naranjo Probability Scale

Categories	Frequency	Per cent
9 + (Highly probable)	0	0
5 - 8 (Probable)	22	70
1 – 4 (Possible)	9	30
0 - (Doubtful)	0	0

Clin Pharmacol Ther 1981;30: 239-45

Table V. Results of the causality assessment using The French Imputation System

Categories	Frequency	Per cent
I 4 – Very likely	8	25
I 3 – Likely	10	32.3
I 2 – Possible	10	32.3
I 1 – Dubious	3	10.4
I 0 – Unlikely (appears excluded)	0	0

Thérapie 1985; 40: 111-8

Table II. Results of the causality assessment using the WHO Causality Categories

Categories	Frequency	Per cent
Certain	9	29
Probable	11	35.5
Possible	10	32.3
Unlikely	1	3.2
Conditional/Unclassified	0	0
Unassessable/Unclassifiable	0	0

- **Certain**
  - Event or laboratory test abnormality, with plausible time relationship to drug intake
  - Cannot be explained by disease or other drugs
  - Plausible response to withdrawal (pharmacologically, pathologically)
  - Definitive event confirmed pharmacologically or phenomenologically (An objective and specific medical disorder or a recognised pharmacological phenomenon)
  - Rechallenge (if necessary)
- **Probable**
  - Event or laboratory test abnormality, with reasonable time relationship to drug intake
  - Unlikely to be attributed to disease or other drugs
  - Response to withdrawal clinically reasonable
  - Rechallenge not necessary
- **Possible**
  - Event or laboratory test abnormality, with reasonable time relationship to drug intake
  - Could also be explained by disease or other drugs
  - Information on drug withdrawal lacking or unclear
- **Unlikely**
  - Event or laboratory test abnormality, with a time relationship with the drug that makes a relationship improbable (but not impossible)
  - Diseases or other drugs provide plausible explanations
- **Conditional / Unclassified**
  - Event or laboratory test abnormality
  - More data for proper assessment needed
  - Additional data under examination
- **Unassessable / Unclassifiable**
  - A report suggesting an adverse reaction
  - Cannot be judged because of insufficient or contradictory information
  - Report cannot be supplemented or verified

Drug Safety 1994;10:93-102

were treated with a monotherapeutic regimen (one anti-infective drug), and 310 (73.64%) were treated with combinations of two or more anti-infective drugs. The most commonly prescribed anti-infective drugs were fluoroquinolones (150 prescriptions recorded in the 6-month period), cephalosporins (111 prescriptions), macrolides (83 prescriptions), aminoglycosides (78 prescriptions) and sulphonamides (54 prescriptions). (Table I)

All recorded AEs were analysed and assessed using 4 scales/methods for AE assessment (WHO, Naranjo scale, ABO scale, French Imputation Method). Depending on the scale that was used, the percentage of AEs that were estimated as *certain/the most probable* varied from 58% (ABO scale), to 29% (WHO), to 25.8% (French Imputation Method) and to 0% (Naranjo scale). (Tables II, III, IV, V)

Anti-tuberculosis drugs caused the highest percentage of AEs in the observed period (38.7%).

AEs that occurred in patients during treatment with anti-infectives affected different system-organ classes. The majority of AEs presented as disorders of the gastrointestinal tract (41.9%), followed by disturbances in laboratory parameters in 22.6% (mostly increased levels of uric acid, AST and/or ALT), hypersensitivity reactions (urticaria) (16.1%), skin disorders (rash) (9.7%), general disorders (weakness) (6.5%) and damaged acoustic nerve/vertigo (3.2%). In the gastrointestinal category, diarrhoea was the most frequent complication (71.4%), followed by nausea and vomiting.

The main causal drugs were anti-tuberculosis agents (rifampicin, isoniazid, pyrazinamide, ethambutol and streptomycin) (38.7%), fluoroquinolones (29%), macrolides (16.1%), cephalosporins (9.7%), sulphonamides (3.2%) and anti-fungal agents (3.2%).

In the group of patients who experienced AEs, 29% had previously experienced adverse reactions to other drugs. In 23 patients (74.2%) the incriminated drug was discontinued, while in 8 patients (25.8%), drug administration was continued because any newly apparent adverse reactions were mild, short-lasting or did not affect patient's health seriously, and it was considered safe to continue with drug administration.

Thirteen cases of anti-infective induced AEs (41.9%) lasted for more than 5 days, while all others lasted fewerless than 5 days (*3 to 5 days* in 5 cases (16.1%), *2 days* in 6 cases (19.3%), *1 day* in 3 cases (9.7%) and a *few hours* in 4 cases (12.9%)).

The outcomes of the AEs were *complete resolution of signs and symptoms* in 20 cases (64.5%), *incomplete resolution of signs and symptoms* in 10 cases (32.3%), consisting mostly of elevated blood transaminase levels at the time of hospital discharge, and *permanent damage* in only one case (3.2%), which had an audiometrically confirmed lesion of the acoustic nerve caused by anti-tuberculosis agents (most likely streptomycin).

The results of the Chi-squared analysis showed that there was no difference in duration of AEs between male and female patients. No statistically relevant difference in outcome of AEs or in previous experience of adverse reaction to drugs between male and female patients was found ($p>0.05$). On the other hand, the Chi-squared test analysis revealed that AEs described as *diarrhoea* and *hypersensitivity reaction* appeared more frequently in males than in females (test value-14.582, $p=0.042$). Although there was no statistically relevant correlation between duration of AEs and patients' sex, a trend was found that AEs in females last longer than in males.

Finally, the results of our analyses indicate that males are at greater risk of experiencing adverse reactions to fluoroquinolones (ciprofloxacin) than females. Female patients are, on the other hand, at greater risk of experiencing adverse reactions to anti-tuberculosis agents than male patients.

DISCUSSION

Many studies have been dedicated to investigation and analysis of AEs, focusing on various aspects such as causality, costs, consequences, hospitalisation, prolonging hospital stay and many others. It is easily noticed that there are great differences between the rates of adverse reactions to drugs reported from these studies. These differences may depend on the definitions used for AE and ADR, the study population, medications used for treatment, the method used for AE/ADR assessment, the vigourintensity with which AEs were sought and the number of drugs administered simultaneously leading to drug interactions [5].

Assessing AEs in inpatients is much easier and much more accurate than in outpatientsbecause because any potential adverse drug reaction is more easily perceived and monitored during hospital stay than in an outpatient setting. However, the probability that many AEs, even in inpatients, happen without being recorded is significant. Some of the effects of ADRs are often attributed to an underlying illness, previous medical history or other causes.

Anti-infectives appear to be first on the list of drugs causing ADRs.[5] This is why we decided to conduct our study in the Center for Pulmonary Diseases, where anti-infectives are frequently used for various previously mentioned indications.

The incidence of AEs in inpatients receiving anti-infectives in our study (7.36%) is within the range reported from other studies.[4,5,12,13] Patients receiving therapy with anti-tuberculosis agents are at the greatest risk of experiencing ADRs. These agents are commonly used in combinations of 4 or 5 drugs (isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin), and although some adverse reactions are more likely to be attributed to one of them or some of them, they were jointly classified as *anti-tuberculosis agents* because it is very difficult to be precise in selecting one of these drugs to incriminate for a specific adverse reaction. Nevertheless, these statistics imply the need to administer these drugs carefully and to monitor patients under therapy regularly to record any AEs promptly and to respond adequately. The second most frequent drug related to AEs in our study was ciprofloxacin, the most frequently prescribed antibiotic in the department.

Adverse events related to drugs in most cases led to prolonged hospital stay (20.13±11.53 days in patients with AEs, 18.16±14.36 in patients total), which significantly increased the cost of hospital treatment.

It should be noted that none of the recorded adverse reactions to specific anti-infectives were unexpected. This raises a dilemma of whether a great portion of these ADRs were preventable or at least predictable.[14]

Another interesting issue that comes across in this study is the causality assessment. The fact that there were great differences in the rating/scoring of AEs recorded in this study shows that the abovementioned scales/sys-

tems for causality assessment are not compatible or even comparable. The Naranjo probability scale is a tool used to determine the likelihood that an AE was caused by the implicated medication. Ten questions are answered and assigned a score of +2 to -2. Where there is insufficient data available, that particular question receives a 0. Based on the Naranjo criteria, each case is scored (< 1 to > 9) and assigned a likelihood of causing an AE (doubtful, possible, probable, highly probable)[9].

Question No.7 assesses whether the drug was detected in the blood at toxic levels, which is not a routine or easily applicable procedure. Question No.4 ("Did the ADR appear with re-challenge?") is also problematic because it refers to re-administration of an incriminated drug to the patient, which few physicians are willing to allow. The same problem applies to question No.8 ("Was the reaction more severe when the dose was increased or less severe when the dose was decreased?"). In our survey, these questions were answered "No" in all cases. This explains why there were no AEs in our study classified as *highly probable* with score of 9 and more. The Naranjo scale is, therefore, applicable primarily to clinical trials and controlled conditions.

The WHO Causality Categories and European Causality Categories (ABO system) are similar, but the WHO Causality Categories are more detailed and therefore more precise (6 levels of adverse event rating) compared to the ABO system (only 3 levels of rating). The French Imputation System seems to be the most detailed and most precise. It has some of the faults already mentioned with the Naranjo Scoring System (drug re-challenging), but its greatest fault is that it is complicated and time-consuming. A rating system based on the French Imputation System consists of 3 phases: chronology (the time relationship between drug administration and the appearance of AE), semiology (the probability that the incriminated drug led to AE and/or other non-drug explanations) and literature grading (data from the literature where similar reactions to the incriminated drug were mentioned).

All of this implies that there is a great need for the development of new causality assessment scales with better sensitivity and specificity. These causality assessment scales (proposed and presently used) can reduce disagreement between assessors, classify relationship likelihood, help in marking individual case reports and be very useful in education and improvement of scientific assessment. On the other hand, it is not possible to expect to reach an accurate quantitative measurement of relationship likelihood, distinguish clearly valid from invalid cases, prove the connection between drug and event or quantify precisely the contribution of a drug to the development of an adverse event, thus increasing causal certainty, using these scales.

The results of this study indicate that physicians or pharmacists interested in investigating AEs related to drugs need to be simultaneously aggressive and cautious at the same timewhen recording and rating/scoring AEs. It seems that choosing adequate anti-infective therapy that is individually adapted to every patient and balanced between safety, efficiency and rationality remains a great challenge for every physician and every pharmacist.

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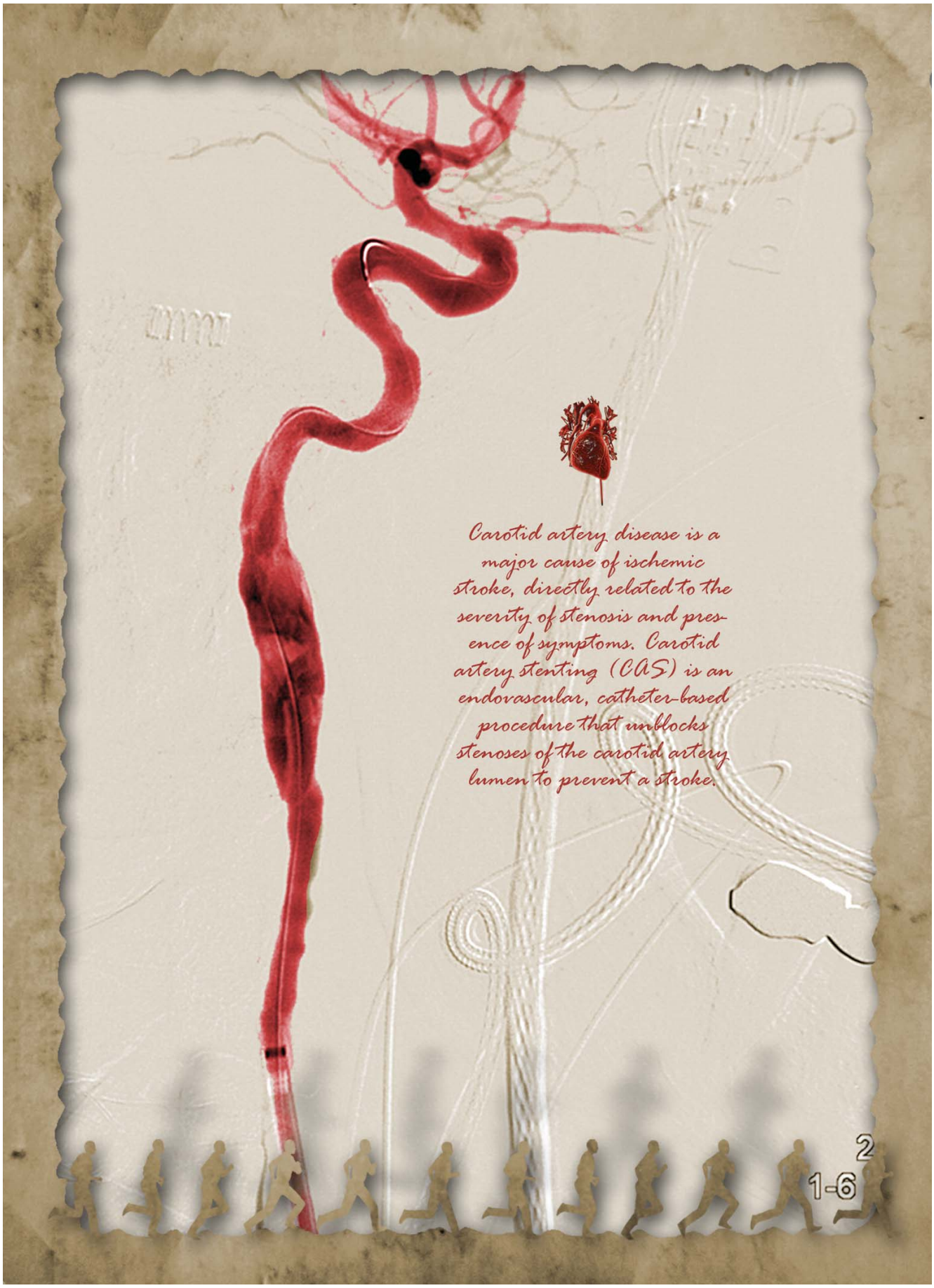
Carotid artery disease is a major cause of ischemic stroke, directly related to the severity of stenosis and presence of symptoms. Carotid artery stenting (CAS) is an endovascular, catheter-based procedure that unblocks stenoses of the carotid artery lumen to prevent a stroke.
1-6 2

CAROTID ARTERY STENTING - OUR EXPERIENCES

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KAROTIDNI STENTING - NAŠA ISKUSTVA

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ABSTRACT

Background: *Carotid artery disease is a major cause of ischemic stroke, directly related to the severity of stenosis and presence of symptoms. Carotid artery stenting (CAS) is an endovascular, catheter-based procedure that unblocks stenoses of the carotid artery lumen to prevent a stroke.*

Objective: *The aim of this study was to analyse outcomes in a series of patients with carotid atherosclerosis (CA) who underwent carotid angioplasty and stenting.*

Methods: *This study was designed as a retrospective cross-sectional study. The report includes 95 patients with carotid atherosclerosis who were treated with carotid angioplasty and stenting. The efficacy of the procedures was estimated by Doppler ultrasound of carotid arteries and by magnetic resonance imaging (MRI) 12 months after the procedure.*

Results*: Observed complications after CAS included carotid artery restenosis in 8 (8,4%) participants and CVI in 9 (9,5%) participants. We concluded that the appearance of CVI depends on the presence of distal protection devices (p =0.003). In contrast to CVI, restenosis of CA after angioplasty and stenting was not related to degree of CA stenosis (p = 0.600) nor to patient age (p = 0.264). Advanced age (p=0.024) and calcified atherosclerotic plaques (p=0.003) were independent predictors of CVI after the procedure.*

Conclusions*: Carotid stenting can be considered the method of choice for the treatment of carotid disease.*

Keywords*: Carotid artery stenting, angioplasty, carotid artery disease*

SAŽETAK

Poreklo. *Bolest karotidnih arterija je glavni uzrok ishemijskog moždanog udara, direktno je povezana sa stepenom stenoze i prisustvom simptoma. Stenting karotidne arterije je endovaskularna procedura bazirana na upotrebi katetera, kojom se `odblokira` suženje da bi se sprečio udar.*

Cilj. *Cilj ove studije je da analizira ishod procedure karotidne angioplastike i stentinga, kojoj je podvrgnuta serija pacijenata sa aterosklerozom karotidne arterije.*

Metod. *Ovo je retrospektivna studija preseka koja obuhvata 95 pacijenata sa karotidnom aterosklerozom podvrgnutih proceduri karotidne angioplastike i stentinga. Efikasnost procedure je procenjena pregledom kolor Dopler ultrazvukom i magnetnom rezonancom, nakon godinu dana.*

Rezultati. *Evidentirane komplikacije posle procedure su - kod 8 (8,4%) učesnika restenoza karotidne arterije a kod 9 (9,5%) pojava CVI. Zaključili smo da pojava CVI zavisi od korišćenja distalne protekcije (p=0.003). Za razliku od CVI restenoza posle procedure nije vezana ni za stepen stenoze arterije (p=0.600) ni za starost pacijenta (p=0.264). Starost pacijenta (p=0.024) i prisustvo kalcifikaovanog aterosklerotskog plaka (p=0.003) su nezavisni prediktori pojave CVI nakon procedure.*

Zaključak. *Stenting se može smatrati metodom izbora za lečenje bolesti karotidnih arterija.*

Ključne reči: *stenting karotidnih arterija, angioplastika, bolest karotidnih artrija*

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INTRODUCTION

Carotid artery disease is a major cause of ischemic stroke, directly related to the severity of stenosis and presence of symptoms (1,2). It is estimated that carotid artery stenosis is responsible for 15% to 20% of all strokes (3). Stroke is the leading cause of functional impairment . More than 20% of surviving patients require institutional care and up to one-third have permanent disability. Even more concerning is the fact that the population is aging, and the number of patients with stroke is correspondingly increasing (1,2).

The primary mechanism of stroke in patients with carotid artery stenosis is embolism of atherosclerotic debris or thrombotic material from the plaque into the distal cerebral vasculature (4). Carotid endarterectomy (CEA) was the first intervention that, in addition to optimal medical therapy, was shown to reduce the risk of further ischaemic events (5). Carotid artery stenting (CAS) is an endovascular, catheter-based procedure, which widens the carotid artery lumen. CAS offers patients a less invasive and less traumatic approach than other procedures that have the same goal (6). Angioplasty is the technique of mechanically widening a blood vessel that has been narrowed or obstructed, typically as a result of atherosclerosis. Angioplasty and stenting are commonly used for the treatment of atherosclerotic stenosis in several arterial territories in the body. These techniques are very effective at reducing the degree of arterial narrowing. The procedures involve the crushing of atherosclerotic plaque material against the vessel wall with a high-pressure balloon and the subsequent placement of a metal mesh tube (stent) to hold this material back, preventing elastic recoil and covering any dissection caused by the angioplasty procedure (7).

Despite some controversy regarding the superiority of carotid angioplasty and stenting compared with carotid endarterectomy, the use of these procedures in clinical practice "exploded" in many parts of the world (8). Nevertheless, one of the limitations of CAS is the potential for embolic stroke caused by dislodging the atheromatous material in arterial plaques (9,10). To prevent stroke, a variety of cerebral protection devices (CPDs) have been developed in recent years. Preliminary results have shown that these devices can significantly reduce the risk of thromboembolic complications during CAS (9,10). However, concerns have been raised regarding protection devices, because their use requires further manipulation and increases the risk and costs of the procedure (11,12).

CAS is still an evolving intervention, and new techniques, such as specialised and miniaturised diagnostic and guiding catheters and guide wires, new stents and new adjunctive therapies (13), are rapidly being developed. A recent systematic review of the literature suggests that the risk of stroke or death after CAS is increased in patients with symptomatic stenosis, in elderly patients, and in patients with hypertension or a history of coronary artery disease (14).

The aim of this study was to analyse outcomes in a series of patients with carotid atherosclerosis (CA) who underwent carotid angioplasty and stenting.

MATERIALS AND METHODS

Our study was designed as a retrospective cross-sectional study. From March 2009 untilto March 2010, 95 patients with carotid atherosclerosis underwent carotid angioplasty and stenting at the Kragujevac Clinical Center in Kragujevac, Serbia.

Before the procedure, all patients received 450 mg of clopidogrel. During the procedure, patients were constantly monitored, and 1 mg of atropine was given (IV). After the procedure, all patents received three months of combination anti-platelet therapy consisting of 75 mg of clopidogrel per day and 100 mg of aspirin (acetylsalicylic acid) per day. Depending on blood cholesterol levels, patients received 20 mg per day of atorvastatin. To be included in the study, patients were required to have symptomatic disease (with neurological manifestations such as TIA, RIND or CVI). Patients also were were also required to have either high-grade stenosis (more than 70%) or one occluded CA with at least 60% stenosis in the contralateral CA. Patients with clopidogrel resistance or with ulcerated/calcified plaques were excluded from this study.

Before the procedure, all patients underwent Doppler ultrasound of the carotid arteries, magnetic resonance imaging (MRI), digital subtraction angiography (DSA) and testing for clopidogrel resistance. Control MRI was performed within 24 hours after CA angioplasty and stenting. Follow-up consisted of control MRI and Doppler ultrasound examination of the carotid arteries one year after the procedure.

We used stents by Boston Scientific and Abbot as well as stents Protégé by AV-3 and Cordis stents. Filters used for distal embolic protection were the EZ by Boston Scientific, the Spyder AV- 3 and the Angiogard by Cordis.

Characteristics of the study population are described with frequencies, means ± standard deviation, and medians. The statistical significance of differences between groups was tested by Fisher's exact probability test (for frequencies) or by the non-parametric Mann–Whitney U test (for continuous variables). Multiple logistic regression analysis was performed to determine independent predictors of any medical complication resulting from the procedure. The differences were considered significant if the probability of the null hypothesis was less than 0.05.

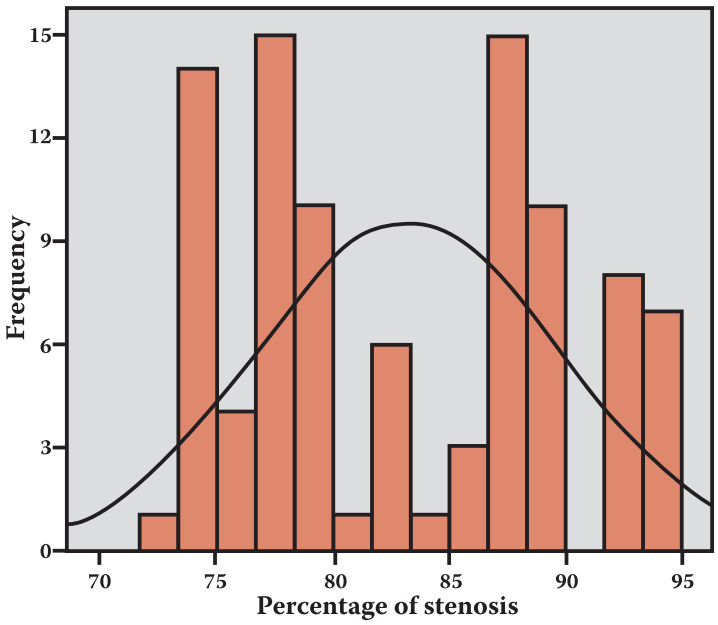
RESULTS

Characteristics of the patient population with identified risk factors are listed in Table 1. As noted above, 95 participants arewere included in the report. Bilateral carotid artery occlusion was observed in 31% of patients, and 16% of patients underwent treatment of both CAs. The degree of carotid stenosis in this study population ranged from 72 to 95% (Graphic 1). After one year of follow-up, the major complications observed in this study were carotid artery restenosis in 8 (8,4%) participants and CVI in 9 (9,5%) participants.

Demographic	
Mean age	71,1 ± 4,433
Men	52 (54,7%)
Women	43 (45,3 %)
Present symptoms	**Number / (%)**
TIA	92 (96,84%)
RIND	43 (45,26%)
CVI	21 (22,11%)
Risk factors	**Number / (%)**
Diabetes mellitus	69 (76.2%)
Hyperlipidemia	94 (98.9%)
Smoking	67 (70.5%)
Coronary artery disease	41 (43.2%)

Table 1. Characteristics of the patient population with identified risk factors

In 45 patients who had atherosclerotic plaques with smooth contours (without ulcerations or calcium), carotid artery stenting was performed without the use of distal protection devices. In this subgroup, we did not observe any instances of CVI during the follow-up period. In the subgroup of 50 patients who underwent CAS with distal protection devices, 9 (18%) patients suffered CVI. Using Fisher's exact test, we concluded that the risk of CVI was related to the use of distal protection devices (p=0.003). In our study population, ulcerated plaques were observed in 24 (25,26%) patients. In patients with ulcerated plaques, CA restenosis at one year was observed in 3 (12,5%) patients, while in the 71 (74,74 %) patients without ulcerations, CA restenosis was seen in 5 (7%) participants. This difference was not statistically significant (p = 0.412 by Fisher's exact test).



Graphic 1. Carotid stenosis in this study population

We also tested the association of CVI (as a neurological complication) with the presence of ulcerated plaques. CVI was observed in 9 (37,5%) patients with ulcerated plaques, while in patients without ulcerated plaques, no cases of CVI were seen. This difference was statistically significant (p < 0.0005 by Fisher's exact test).

Calcified atherosclerotic plaque was identified in 12 (12,63%) study participants. Of these participants, CA restenosis within the first year after the procedure was observed in 3 of these(25%) patients. In the 83 (87,37%) patients without calcified plaques, restenosis was observed in 5 (6%) patients. This difference was not statistically significant (p = 0.061 by Fisher's exact test).

We also attempted to establish if patients with calcified plaques had a higher risk of CVI after CAS. In the subgroup of 12 patients with calcified plaques, 8 (66,7%) suffered CVI within one year. In the 83 patients without calcified plaques, only 1 (1,2%) patient suffered CVI. This was a statistically significant difference (p < 0.0005 by Fisher's exact test).

The relation between CA restenosis after CAS and the degree of stenosis before the procedure was also tested. We found that restenosis of CA and degree of pre-procedural CA stenosis were not significantly related (Mann-Whitney U Test, p=0.600). Patient age was also not significantly related to the risk of CA restenosis (Mann-Whitney U Test, p=0.264).

Patients who suffered CVI had a higher degree of pre-procedural CA stenosis than patientsthose who did not experience this complication. The median percentage of CA stenosis in patients who suffered CVI was 90%. Patients who did not have CVI had a median CA stenosis percentage of 80%. This difference was statistically significant (Mann-Whitney U Test, p=0.001). Patients who experienced CVI also were were also significantly older, with a median age of 78 years versus 71 years in patients who did not have CVI (Mann-Whitney U Test, p=0.0005).

The effect of clinical characteristics on the subsequent development of medical complications was analysed by logistic regression. Multiple logistic regression analysis was performed to determine the independent predictors of any post-procedural medical complication. RIND (OR 16.061, 95% CI 1.767-145.996, p = 0.014) and calcified plaques (OR 19.661, 95% CI 1.616 – 239.236, p=0.019) significantly increase the risk of CA restenosis after CAS. Multiple logistic regression analysis revealed that advanced age (p=0.024) and presence of calcified plaques (p=0.003) were independent predictors of the risk of developing CVI as a complication of CAS. In terms of age, the OR for advanced age was 1.696 (95% CI 1.072 - 2.685), implying that every year of increasing age increases the risk of CVI by 70%. The OR for CVI in patients with calcified plaques was 106.390 (95% CI 5.058- 2237.887), suggesting that the presence of calcified plaques increases the risk for CVI by a factor of 106.

DISCUSSION

Carotid artery balloon dilation with stenting with balloon dilation is a minimally invasive treatment for CA stenosis, possible cause of cerebrovascular insult . Several elements are required for a successful CAS procedure, including adequate equipment such as stents, a well-trained team of doctors, and good cooperation with neurologists. Neurologists should document symptoms such as transient ischemic attack and confirm the presence of stenosis using special diagnostic examinations. Studies of carotid stents began in 2001. Documented complications include stroke; the mortality rate ranges from 0% to 7.4%. This variation is likely related to differing patient ages and comorbidities in different published series (15).

In the present study, the incidence of risk factors, such as abnormal lipid profile and diabetes mellitusmellitus, was high. Coronary artery disease was also observed in most of our study patients. The treatment of patients with CA stenosis and medical comorbidities is complex and requires long-term follow-up. Stent placement and balloon angioplasty is only treatment of consequence, and the origin of atherosclerosis is complex and requires serious access.

The use of distal embolic protection devices during carotid stenting is necessary to increase the safety of this procedure by reducing the possibility of migration of particles from ruptured plaques. A recent meta-analysis by Kastrup supports the use of cerebral protection devices, showing a reduction in neurologic events from 5.5% to 1.8% with the use of cerebral protection devices (16).

Our study showed a statistically significant relationship between the presence of ulcerated plaques and the risk of cerebrovascular insult after the CAS procedure. Several reports have stressed the fact that high-risk morphology plaques have a high propensity to embolise and cause stroke (17).

Choosing the proper stent for the type of lesion being treated reduces the risk of CA restenosis. In our study, the grade of CA stenosis did not predict for the risk of restenosis, suggesting that the selection of stents and balloon angioplasty devices was adequate.

Finally, there still is a need to identify specific risk factors for the development of restenosis after CAS. Some studies have identified advanced age as a potential risk factor for the development of a restenosis; however, the definitive role of these factors still needs should be elucidated in larger trials (18).

Our study has showed that rates of restenosis after stenting were not significantly correlated to patient age. However, the risk of cerebrovascular insult after stenting was higher in older patients. Because advanced age has also been associated with a higher frequency of neurologic complications after CAS, the elderly should generally be considered as high-risk patients for CAS (19, 20).

The relatively low complication rate in our study points to good pre- and post- procedural antiplatelet treatment as well as adequate drug treatment of hypercholesterolemia. It also points to the positive attitude of most of our patients related to the reduction of their risk factors .

CONCLUSIONS

Carotid stenting is thea treatment of choice for carotid disease. Key factors for the success of the procedure are prompt diagnosis of the presence of atherosclerosis in the carotid arteries, adequate preparation of the patient before the procedure, proper stent selection and adequate positioning within the arteriosclerotic lesions. In addition, postprocedural support for risk factor reduction also plays a significant role in lowering the rates of restenosis and cerebrovascular complications.

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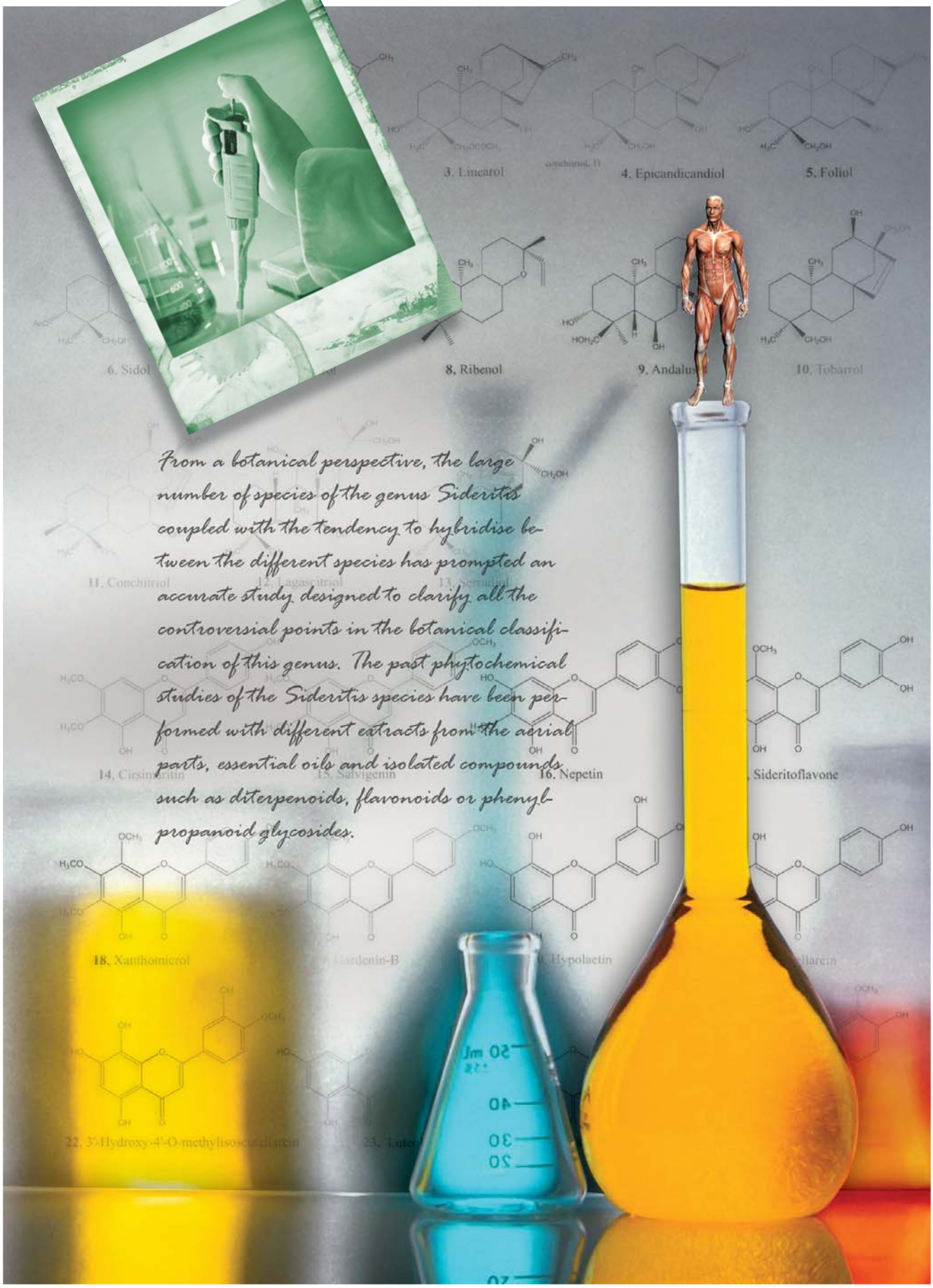

From a botanical perspective, the large number of species of the genus Sideritis coupled with the tendency to hybridise between the different species has prompted an accurate study designed to clarify all the controversial points in the botanical classification of this genus. The past phytochemical studies of the Sideritis species have been performed with different extracts from the aerial parts, essential oils and isolated compounds such as diterpenoids, flavonoids or phenylpropanoid glycosides.

SUMMARY OF THE PHYTOCHEMICAL RESEARCH PERFORMED TO DATE ON SIDERITIS SPECIES

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PREGLED DOSADAŠNJEG FITOHEMIJSKOG ISPITIVANJA SIDERITIS VRSTA

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ABSTRACT

From a botanical perspective, the large number of species of the genus Sideritis coupled with the tendency to hybridise between the different species has prompted an accurate study designed to clarify all the controversial points in the botanical classification of this genus. The past phytochemical studies of the Sideritis species have been performed with different extracts from the aerial parts, essential oils and isolated compounds such as diterpenoids, flavonoids or phenylpropanoid glycosides. These investigations have justified the traditional uses for these plants and provided direction for new pharmacological research.

In recent years, other compounds such as iridoids, coumarins, lignans and phenylpropanoid glycosides have also been isolated and identified. The results have shown that essential oils act as good antimicrobial agents against both Gram-positive and Gram-negative bacteria and against the Candida albicans fungus. Diterpenoids have shown antimicrobial, anti-inflammatory and antifeedant activity, and flavonoids are active as anti-ulcerous, anti-inflammatory and antioxidant agents. Future research should focus on the pharmacological activity of these isolated compounds to find new active principles and identify their mechanisms of action. In addition, it would be interesting to investigate new pharmacological activities apart from those used in traditional medicine; recent studies have shown that diterpenes and some diterpene derivatives act as effective anti-HIV and antiproliferative agents.

Keywords: *Sideritis genus, traditional use, essential oils, flavonoids, antimicrobial, anti-inflammatory activity*

SAŽETAK

Veliki broj vrsta u okviru roda Sideritis, njihova izražena tendencija međusobnog ukrštanja razlog je sprovođenja obimnog istraživanja u cilju razjašnjavanja kontraverznih činjenica vezanih za botaničku klasifikaciju ovog roda. Fitohemijske studije Sideritis vrsta obuhvataju izučavanja različitih ekstrakata nadzemnih delova, etarskog ulja i izolovanih jedinjenja kao što su diterpenoidi, flavonoidi ilifenilpropanoidni glikozidi, kao jedinjenja nosioci aktivnosti. Ova istraživanja omogućavaju da se sprovođenjem farmakološki osmišljenih eksperimenata opravda tradicionalna primena ovih biljaka, kao i da se opravda eventualna upotreba ovih biljaka i njihovih ekstrakata u tretmanu nekih oboljenja za koje tradicionalno nije poznata upotreba biljaka ovog roda.

U poslednjih nekoliko godina, brojna u okviru sprovedenih iscrpnih istraživanja, jedinjenja kao što su iridoidi, kumarini, lignani, kao i fenilpropanoidni glikozidi izolovani su i identifikovani u ekstraktima ovih biljaka. Rezultati su pokazali da eterska ulja deluju kao dobri antimikrobni agensi i da deluju na gram pozitivne i gram negativne bakterije i gljivice Candida albicans . Diterpenoidi su pokazali antimikrobno, antiinflamatorno delovanje, a doprinose i odbrani biljaka od biljojeda. Flavonoidi deluju kao anti-ulcerozni, antiinflamatorni i antioksidativni agensi. Buduća istraživanja trebalo bi da buduusmerena na ispitivanja farmakoloških aktivnosti izolovanih jedinjenja, u cilju pronalaženja novih aktivnih principa i objašnjavanja mehanizma njihovog delovanja. Pored toga, istraživanja ne bi trebalo ograničiti samo na potvrdu tradicionalne upotrebe biljaka roda Sideritis, već bi bilo zanimljivo istražitimogućnost njihove upotrebe za nova indikaciona područja. Nedavne studije su pokazale da diterpeni i neki diterpenski derivata pokazuju anti HIV delovanje, a poseduju i antiproliferativni efekat.

Ključne reči: *Rod Sideritis, tradicionalna primena, eterska ulja, flavonoidi, antimikrobna, antiinflamatorna aktivnost*

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INTRODUCTION

The genus *Sideritis* (Lamiaceae) comprises more than 150 perennial and annual vegetal species widely distributed in the Mediterranean area, together with the Canary and Madeira islands. It is a controversial botanic genus with a complex taxonomical classification due to the high number of hybridisations that occur between species; their study requires substantial and deep research experience.

The results of numerous preliminary investigations of plants belonging to the genus *Sideritis* L. revealed plant-derived substances of particular pharmacological and nutritional interest. So far, a wide variety of biological activities of the *Sideritis* species have been reported: anti-inflammatory, anti-ulcer, analgesic, antimicrobial and antifungal (1-5), antifeedant (6), anticataract (7), immunomodulating (8) macrophage NOS-2-expression inhibiting (9) and hypoglycaemic (4). Recently, aldose reductase-inhibiting activity (12) and antiproliferative, anticholinesterase and selective oestrogen receptor modulator-like effects have been reported (11-13). The previous studies of the *Sideritis* species reported the presence of flavonoid aglycones and glycosides, phenolic acids, di- and triterpenoids, fatty acids, coumarins and iridoid glycosides (2, 10, 12, 14-28). Most of the studies on the *Sideritis* species attributed the previously cited biological activities mainly to phenolic compounds. Recently, several articles have been published describing the connection between the phenolic constituents and pharmacological activity in various *Sideritis* species (10, 14). Rios et al. (19) reported that flavonoids were reducing agents able to interact with free radical species (relevant to the autoxidation mechanism) and could prevent the generation of inflammatory mediators. The composition of the various *Sideritis* species essential oils has also been studied exhaustively (1, 20).

The genus *Sideritis* is represented in Serbia by one species only, *S. montana* L. (21), but because of its prooxidant properties, this plant has not been used in traditional medicine (22). *S. scardica* Griseb. (ironwort, mountain tea) is a plant endemic to the Balkan Peninsula, belonging to the *Empedoclia* section. The aerial parts of "mountain tea" are traditionally known for their anti-inflammatory, antimicrobial, antibacterial, antirheumatic and gastroprotective properties. *S. scardica* is used as a loosening agent in bronchitis and bronchial asthma and against the common cold and lung emphysema, as well as widely used in the treatment of inflammation, gastrointestinal disorders and coughs and as an active constituent of dietary supplements for the prevention of anaemia (23). All biological activities previously cited in the literature have been mainly attributed to the phenolic content of this plant (15).

The goal of this review is to provide a comprehensive overview of the botanical, phytochemical and pharmacological aspects of the genus *Sideritis*.

BOTANICAL ASPECTS

Sideritis L. (Lamiaceae) includes approximately 150 species of annual and perennial plants, distributed mainly in the Mediterranean region and in the moderate zones of Asia. The *Sideritis* L. genus belongs to the family of Lamiaceae Lindl. (Labiatae Juss.), which is one of the most common and diverse plant families of the world. The genus of the Lamiaceae families exists in different altitudes and habitats, ranging from the North Pole to the Himalayas and from Northeast Asia to Hawaii, Australia, Africa and America. However, its main habitat is the Mediterranean area. The taxonomy of the genus *Sideritis* is rather complex because of interspecies hybridisation, and therefore, it has not been satisfactorily resolved. Based on pollen features, Heywood has divided the Labiatae into two subfamilies, Lamioideae and Nepetoideae. Plants from the subfamily Lamioideae are characterised by a low concentration of essential oils, a lack of rosmarinic acid and the presence of iridoid glycosides; Nepetoideae plants are rich in essential oils, contain rosmarinic acid in various percentages and lack iridoids.

The position of the genus *Sideritis* is illustrated in Figure 1. This genus is divided into two subgenera, *Sideritis* and *Marrubiastrum,* formed by the European and Macaronesian species, respectively. The subgenus *Marrubiastrum* (Lamioideae: Lamiaceae) represents one of the most species-rich Macaronesian endemics, containing 24 (23 extant) perennial species distributed among the 10 islands of the Madeiran and Canary Island archipelagos. These plants display a wide range of morphological diversity and are found in all ecological zones in the islands. Growth forms include suffrutescent perennials, chasmophytic (cliffdwelling) rosette plants, and large arborescent shrubs. The Macaronesian floristic region comprises the five Atlantic Ocean archipelagos of the Azores, Madeira, Selvagen, Canary, and Cape Verde Islands and is situated between 15° and 40° north latitude. While some of the Macaronesian islands are comparable to their Pacific counterparts in their extreme isolation, others are much closer to continental source areas, with only 100 km separating the island of Fuerteventura from mainland Africa. The second, much larger subgenus, *Sideritis*, contains approximately 125 species of both annuals and perennials, most of which are suffrutescent but none truly woody, with a centre of distribution in Mediterranean Europe and northern Africa. It comprises four sections, two of which, *Hesiodia* and *Burgsdorffia*, are small groups containing only annual species distributed widely throughout the Mediterranean and Central Asia. The remaining two continental sections, *Sideritis* and *Empedoclia*, contain suffrutescent perennials with centres of diversity in the western Mediterranean area (especially the Iberian Peninsula) and the eastern Mediterranean (Balkans, Turkey, Syria), respectively (24, 25). The continental taxa have a Mediterranean centre of distribution, although a few species

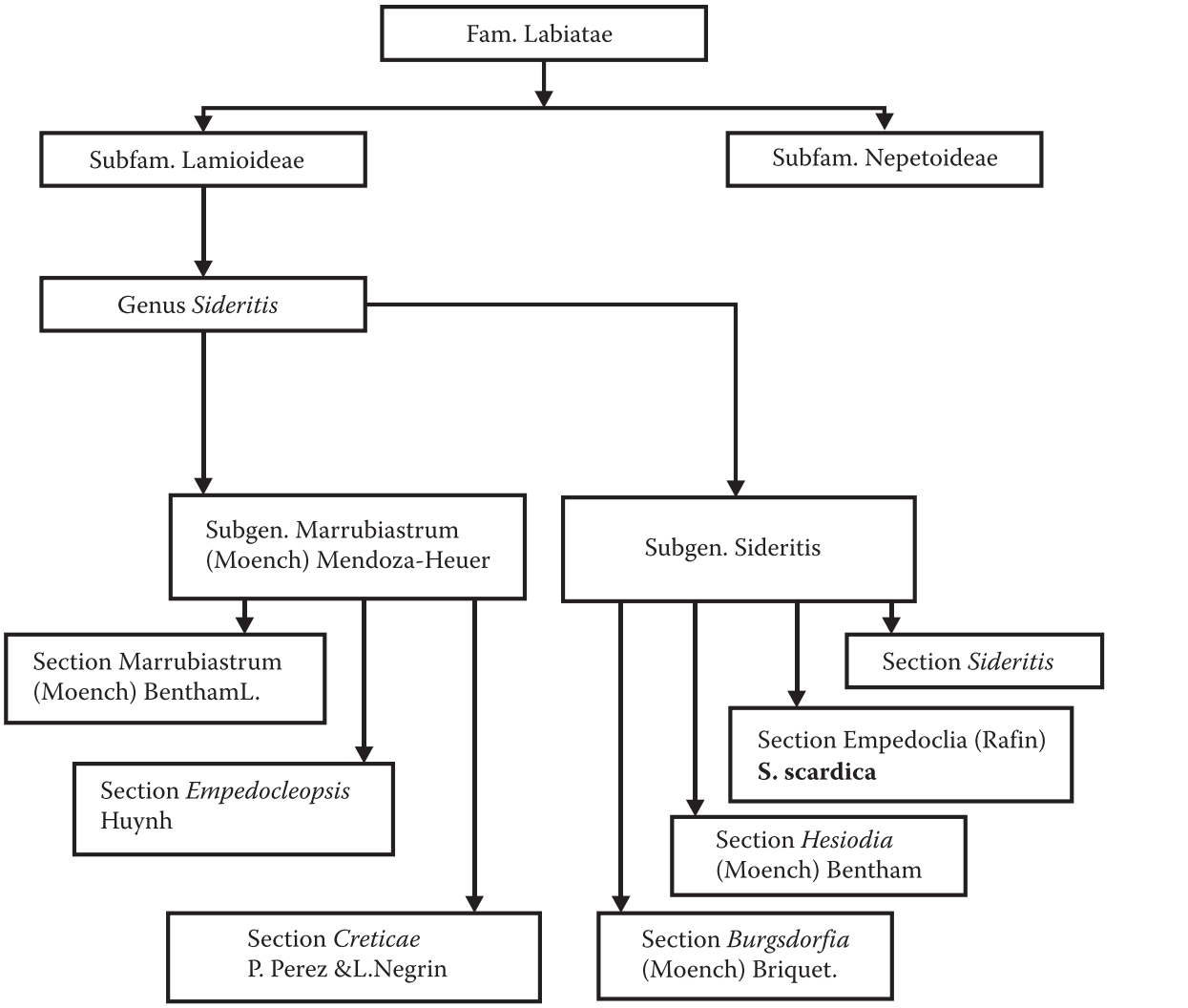


Figure 1. The "botanical tree" of the *Sideritis* genus and the position of *S. scardica*

of *Sideritis* are also found in the Balkans, Eurasia, and the Mideast. The section *Sideritis* contains taxa from the western Mediterranean regions of southern Europe and northern Africa. The species forming the section *Empedoclia* are found in the countries of the eastern Mediterranean (Greece, Turkey) and the Mideast (Israel, Lebanon, Palestine, Iraq, Syria). The morphological characteristics that distinguish the two sections include dentate/spinose bracts and tetracolpate pollen in the section *Sideritis* vs. entire bracts and sixpantocolpate pollen in the section *Empedoclia*.

In addition, it is important to point out that the *Sideritis* genus contains a large number of endemic species: 46 species, 12 subspecies and two varieties grow in Turkey, with 36 species, 10 subspecies and two varieties of them being endemic (77% endemism ratio). Twenty-five *Sideritis* species grow in Morocco, 16 of which are endemic. The Iberian Peninsula and the Baleares Islands contain 49 *Sideritis* species, of which 36 are endemic; in the Canary Islands, this genus is represented by 19 endemic species.

TRADITIONAL USES

The aerial parts of plants from the genus *Sideritis* are widely used as a popular decoction or infusion, orally or topically administered. Most of the medicinal uses of *Sideritis spp.* are limited to folk medicine, although it is worth noting the increasingly frequent presence of *Sideritis spp.* in the herbal remedies market and the increasing number of prescriptions that contain the *Sideritis* species. In Mediterranean folk medicine, aqueous preparations of plants of this genus are considered as antioxidant, anti-inflammatory, anti-ulcerative, antimicrobial, vulnerary, antispasmodic, anticonvulsant, analgesic and carminative agents (26). In Spanish folk medicine, some *Sideritis* species are known as "rabo de gato" or "zahare ña". This last name seems to be the oldest common way to name some *Sideritis* species, such as *S. hirsuta* or *S. arborescens*. The different plant parts have different modes of use; the infusion of the aerial parts has been used for its gastroprotective properties, whereas the decoction of the leaves

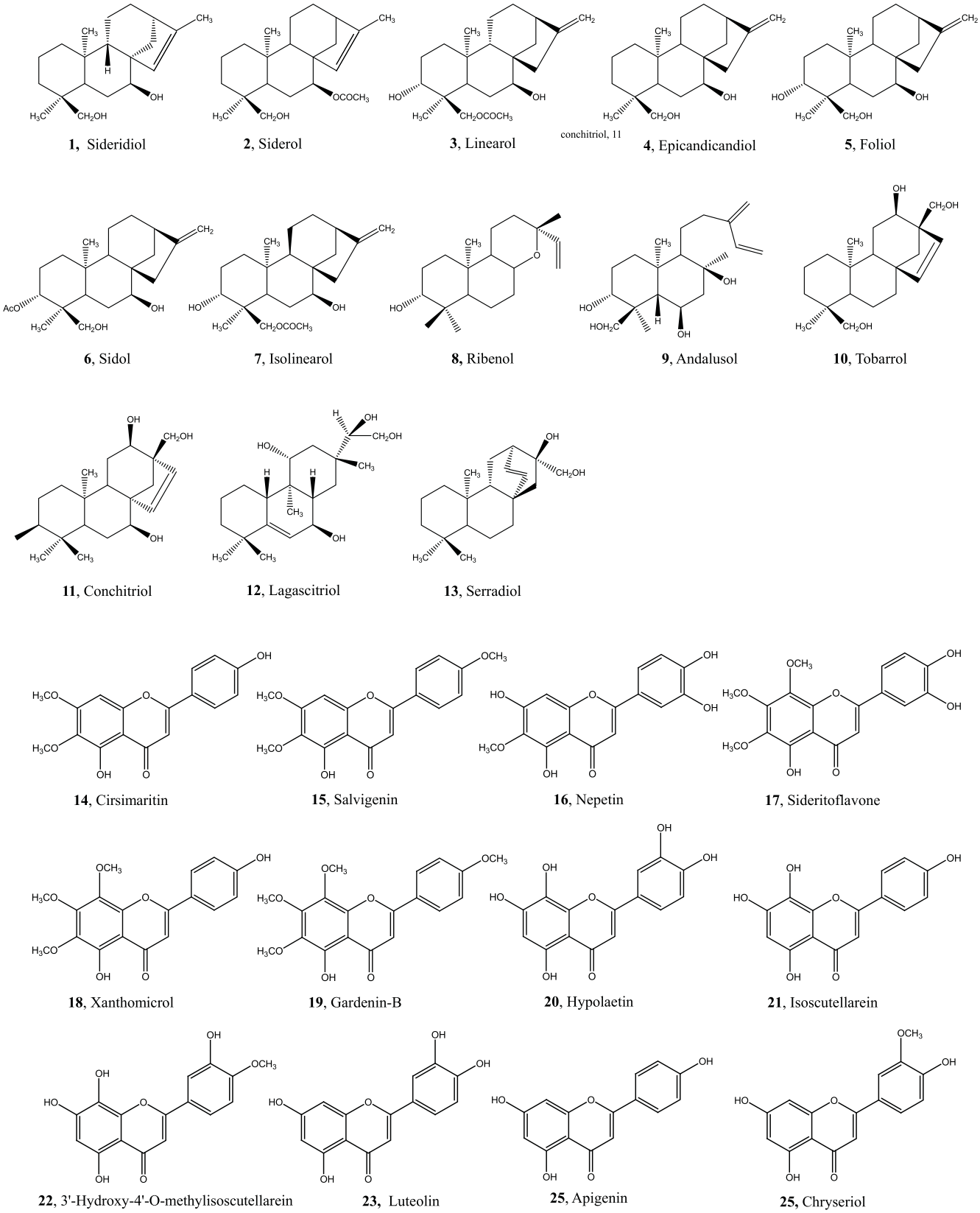
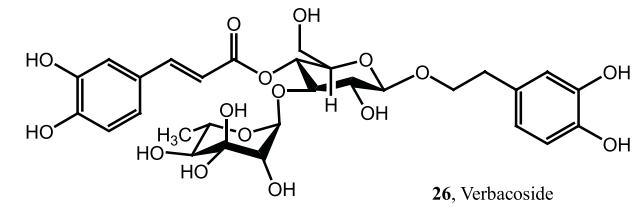
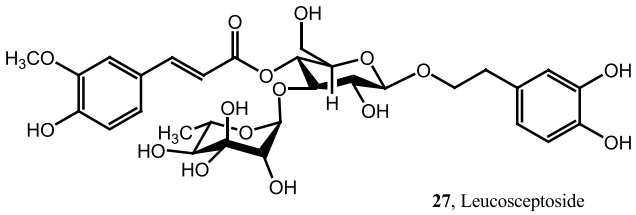
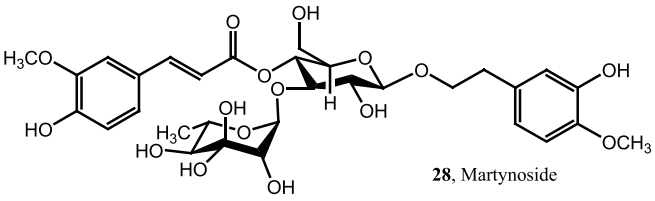


Figure 2. The structures of compounds isolated from the *Sideritis* species







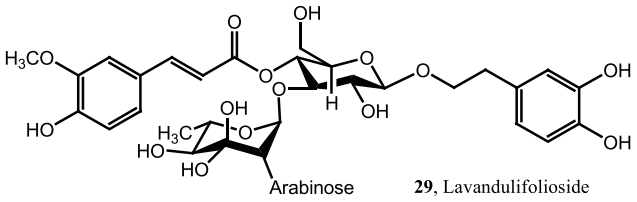


Figure 2. The structures of compounds isolated from the *Sideritis* species

has been employed as an anti-inflammatory and anti-rheumatic preparation. Moreover, the water extract of the stalks has been employed externally for disinfecting and healing wounds and burns. In Turkey, where the *Sideritis* species are known as "dag cayi, yayla cayi", and in Greece, the aerial parts of these plants are widely used to prepare herbal remedies and traditional teas against gastrointestinal disorders such as stomach ache, indigestion and flatulence, to alleviate the symptoms of common colds including fever, flu, sore throat, and bronchitis and a tonic and diuretic remedy. The *Sideritis* teas are named in a variety of ways, but they are commonly known as "mountain tea" because some species grow in the high mountain areas.

The plants of this genus are widely used in traditional medicine in Greece and Europe due to their anti-inflammatory, anti-rheumatic, anti-ulcer, digestive and antimicrobial properties, which are attributed to their phenolic and terpenoid content (1, 2, 27). Differences in uses have been observed among the *Sideritis spp*. and the regions where they grow, depending on the plants' properties. Some of them are widely used in folk medicine in the Balkan countries as herbal teas for the treatment of inflammations, coughs and gastrointestinal disorders. In the Taurus Mountains of Turkey, a poultice prepared with boiled leaves of *S. psidica,* barley flour, grated onion and pine tar is applied on the abdomen as a plaster to treat abdominal pain. Furthermore, in Turkey, *S. syriaca* is used as a diuretic agent and to relieve coughs. An infusion prepared from the leaves and flowers of *S. leptoclada* is used because of its expectorant effect and to fight the common cold in the inner west of Anatolia (Turkey) (28). The endemic species *S. trojana* is one of the most demanded and consumed medicinal plants in bazaars by Turkmens and villagers who live in the sacred mountain called Mount Ida, "the mountain of the Goddess", for the treatment of throat, peptic and chest illnesses (29). In Madeira and the Porto Santo Islands, an infusion of *S. candicans* ("herva branca" or "selvageira") is taken to treat bronchitis and intestinal diseases. An ethnopharmacological study of the plants growing in the Alto Tirreno Cosentino area, in the Calabria region (Southern Italy), revealed that the native people use *S. syriaca* leaves to stop the bleeding from a cut. In Bulgaria, the infusion of the aerial parts of *S. scardica* is employed as an expectorant for the treatment of pulmonary emphysema and angina pectoris (30). Infusions and decoctions of the leaves and flowers of *S. congesta*, *S. libanotica* and *S. psidica* are very popular for treating coughs, hypertension and the "worm in the eyes" syndrome (it is traditionally believed that pains in the eyes are caused by worms with black heads). In the region of Níjar-Cabo de Gata, located in southeastern Spain, *S. granatensis* infusions are used effectively against lower back pain and eye infections and as appetite stimulants. Moreover, a mix of *S. granatensis* and thyme infusion with an added tablespoon of oil is employed as a remedy for hepatic affections. To relieve the cramping that occurs commonly during menstruation, infusions or decoctions from the aerial parts of *S. foetens*, prepared alone or mixed with *S. angustifolia*, are traditionally used in Spain. And infusions from the species *S. tragoriganum*, one of the best-known medicinal plants found in Valencia-Alicante, Spain, are an effective treatment for healing wounds, digestive disorders and urinary tract infections as well as for making gargles and for ocular and dermical washes.

Many species of the genus growing in the western Mediterranean region, especially in Spain and Portugal, have long been studied to determine their chemical constituents, and the phytochemical data have been successfully used in chemotaxonomic studies. Although Turkey is the second largest source of the genus *Sideritis*, except for reports on essential oils, phytochemical information on the genus is still scant (31, 32).

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION

Many chemical constituents have been identified in the *Sideritis* genus, such as terpenes, flavonoids, essential oils, iridoids, coumarins, lignans and sterols. The activity of these plants is mainly due to their flavonoid and terpenoid contents.

The *Sideritis* species are known for the presence of diterpenes of a remarkable structural variability. Sesquiterpenes and triterpenes are not common. *S. italica* was the first genus investigated in the context of the presence of diterpenes; the diterpenoids, sideridiol (**1**) and siderol (**2**), were the first isolated, and their structures were elucidated. The diterpenoids analysis led to the conclusion that kaurene diterpenes were exclusively present in the species occurring in the eastern and central Mediterranean area (Turkey, Greece and Italy). Not only kaurenes but also isokaurene derivatives are found in *Sideritis* species from Greece, whereas the species from Spain and the Canary Islands are rich in kaurenes, but isokaurenes are less common. Species growing in the western Mediterranean area and Macaronesic regions contain diterpenes with different carbon skeletons (*ent*-kaurane, labdane, atisene, pimarane, beyerane, trachilobane and rosane). In the literature, it is reported that the *ent*-kaurene compounds of *S. siplylea* Boiss include siderol (**2**), linearol (**3**), epicandicandiol (**4**) and foliol (**5**) in addition to other compounds. The most common kaurene diterpene derivatives found in the *Sideritis* species are foliol (**5**), sidol (**6**), linearol, sideridiol, and isolinearol (**7**). Aside from the compounds previously mentioned, the most common are labdane (ribenol, **8**, andalusol, **9**), beyerane (tobarrol, **10** and conchitriol, **11**), rosane (lagascatriol, **12**) and atisane (serradiol, **13**). The *ent*-kaurene diterpenes have been studied in microbiological transformation reactions, and a hydroxyl group appears to exert an inhibitory effect on transformations involving oxidation at C-19 (12). Tobarrol is present only in traces in some of the species studied. As expected, there were only traces of terpenoids in the methanol extract. In general, the diterpenoid content was higher in the hexane extracts than in the methanol extracts, andalusol having a higher proportion in both the hexane and the methanol extracts. The previously described anti-inflammatory activity of andalusol justifies the popular use of these species, especially *S. pusilla* and *S. leucantha* ssp. *incana* var. *meridionalis,* as anti-inflammatory agents. The diterpenoids serve as chemotaxonomic markers (33).

Squalene, the acyclic precursor of the triterpenes and sterols, has been identified in *S. argosphacelus* var. spicata*, S. discolor* and *S. lotsyi* var. *mascaensis*. The triterpene derived from this, rhoiptelenone, was confirmed to be present in *S. macrostachya*. The triterpene rhoiptelenol has been isolated from *S. candicans* var. *eriocephala, S. lotsyi, S. discolor, S. lotsyi* var. *mascaensis, S. tenoi* and *S. soluta*. A mixture of α- and β-amyrin has been found in *S. argosphacelus* var. *spicata*, S. *discolor, S. kuegleriana, S. lotsyi* var. *mascaensis* and *S. tenoi.* The corresponding C- 28 acids, ursolic and oleanolic acids, have been isolated from *S. discolor, S. candicans* var. *eriocephala, S. lotsyi* var. *mascaensis* and *S. soluta*, and their acetates have been isolated from *S. kuegleriana* (25). Other pentacyclic triterpenes, erythrodiol and lupeol, have been obtained from *S. discolor* and S. *argosphacelus* var. *spicata*, respectively.

The genus Sideritis is a rich source of flavonoids. A correlation between the flavonoid type and the geographical distribution of *Sideritis* species has been established: 5,6,7-trioxygenated flavones (cirsimaritin, **14**, salvigenin, **15** or nepetin, **16**) are predominant in Macaronesian species, whereas the presence of 5,6,7,8-tetraoxygenated flavones (sideritoflavone, **17**, xanthomicrol, **18** or gardenin-B, **19**) is higher in Mediterranean species. The distribution of the flavone glycosides in the genus *Sideritis* is of interest from a taxonomic viewpoint. In particular, 8-OH-flavone glycosides are characteristic of some sections of *Sideritis*. The 7-allosylglycosides of hypolaetin (**20**), isoscutellarein (**21**), and 3'-hydroxy- 4'-O-methylisoscutellarein (**22**) were also reported from different *Sideritis* species, such as *S. hypsophila*, *S. javalambrensis* and *S. mugronensis*. In contrast to the sections of Empedocleopsis and Creticae, the section Marrubiastrum was reported as one of the richest *Sideritis* species in 8-OH-flavone glycosides. Among the Ibero-North African *Sideritis* species, the species of the section *Sideritis* (Eusideritis Bentham) were also characterised by the accumulation of the 7-glycosides of 8-OH-flavones (isoscutellarein, hypolaetin and their methyl ethers). Moreover, the presence of 6-OH-flavone and 8-OH-flavone 7-glycosides is also a characteristic feature of the Lamiaceae, Scrophulariaceae and chemically related families. It was observed that *S. brevibracteata* produces a good anti-inflammatory, antinociceptive, antioxidant and AR inhibitory herbal tea, as among the 17 species (18 taxa) of *Sideritis* investigated, this species was found to be the richest in 8-OH-flavone glycoside content (10, 16). *S. foetens*, *S. luteola*, *S. leucanthu* var. *incana* represent a group of species with a higher content of polar flavonoids, such as isoscutelarin-7-glucoside and hypolaetin-8-glucoside, which are well documented for their anti-inflammatory and anti-ulcer activity, and luteoline (**23**), which shows vasodilatory activity (34). Furthermore, the investigation of the Macedonian *Sideritis* species (*S. scardica, S. raeseri*) illustrated the presence of a complex profile of hydroxycinnamic acids, phenylethanoid glycosides and both acetylated and nonacetylated flavonoid 7-*O*-glycosides. Two types of flavones, 8-OH (hypolaetin and isoscutellarein and their methoxy derivatives) and 5,7-OH (apigenin and luteolin), have been confirmed. All the flavonoid glycosides detected were 7-*O*-allosyl-(1,2)-glucoside derivatives, 5,8-dihydroxyflavones with a different substitution in the B-ring. Differences in the phenolic profile of hydroxycinnamic acid and flavonoid 7-*O*-glycosides were found between *S. scardica* and *S. raeseri*. Feruloylquinic acid was detected only in the methanol extract of *Sideritis raeseri* (wild growing). Flavonoid 7-*O*-diglycosides were not detected in the methanol extract of *Sideritis scardica* (wild growing) (15). The identification of the flavonoids in wild-growing populations of *S. scardica* and *S. raeseri* in this central Balkan region and the presence of two types of flavones, 8-OH (hypolaetin and isoscutellarein and their methoxy derivatives) and 5,7-OH (apigenin, **24**, and chryseriol, **25**), have been confirmed, and the possibility of distinguishing between the two studied species (*S. scardica* and *S. raeseri*) has been suggested (27).

Many studies have been performed on the chemical composition of essential oil from the *Sideritis* species. Although the Lamiaceae family is well known for its essential oil content, the *Sideritis* species cannot be considered rich in essential oil. But the correlation between the oil yield and the main group of constituents has been established – the higher the essential oil yield, the higher the monoterpene hydrocarbon content. The composition of the essential oils of several species of *Sideritis* was investigated by gas chromatography–mass spectrometry (GC–MS) and gas chromatography (GC) techniques. A large number of studies about the essential oil compositions in *Sideritis* now explain the polymorphism among the populations and the existence of new species, chemical varieties and hybrids. Several *Sideritis* essential oils are characterised by high contents of monoterpene hydrocarbons with α-pinene, β-pinene, sabinene, myrcene or limonene as the main compounds. An important number of sesquiterpene hydrocarbons, particularly δ-cadinene and β-caryophyllene, are normally also found. Other essential oils are rich in oxygenated sesquiterpenes, such as α-cadinol, bisabolol or muurol-5-en-4β-ol, as the main compounds, and finally, diterpene compounds have also been found in *Sideritis* essential oils. The presence of diterpenes as volatile compounds has been described in other genera, such as *Cistus*, *Wollemia*, *Juniperus* and *Helichrysum*, characterised by the same feature that occurs in *Sideritis,* with a large number of these compounds present in the aerial part extracts. Their existence is interesting because it offers another tool for a better understanding of the chemotaxonomic relationship among *Sideritis* species. Analysis based on the presence or absence of volatile diterpene compounds may be promising as chemotaxonomically important for the whole genus. Turkey's endemic species *S. bilgerana*, *S. ozturkii* and *S. cilicica* were rich in the monoterpene hydrocarbons α- and β-pinene. *S. cilicica* has been shown to have relatively high content of β-phellandrene (35). In the group of *Sideritis* species rich in sesquiterpenes, the main constituents have been found to be β-caryophyllene, D-germacrene and calamene (*S. curvidens, S. montana*). Oxygenated derivatives are not common as main constituents in the *Sideritis* species. Oxygenated monoterpenes, along with thymol, are characteristic constituents in *S. romana*. Oxygenated sesquiterpenes predominate in the essential oils of *S. phlomoides* and *S. taurica*. The main constituents of *S. congesta* and *S. argyrea* essential oils are α- and β-pinene, while limonene is the major constituent of *S. perfoliata* essential oil. *S. condensata* provides an essential oil with high proportions of β-caryophyllene and α-pinene (36). *S. perfoliata* and S. *dichotoma* essential oils are rich in diterpenes (37). Monoterpene hydrocarbons have also been reported as a main constituent in the *Sideritis* species growing in Greece and in some Spanish species. In the essential oil of the endemic Spanish species *S. ibanyezii*, sabinene and α-pinene have been found as main compounds (20, 38). Because there are many reports on the chemical composition of *Sideritis* species, it may be useful to present Table 1, which shows both the yields and the main components, and the species are presented in alphabetical order.

Table 1. Main constituents and yields of the essential oils of Sideritis sp., as previously reported

Concerning the other constituents, the recently performed investigation confirmed that iridoids are rare compounds in these species, as are coumarins and lignans. The presence of fatty acids in the seed oil composition has been reported, assuming linoleic acid as the main fatty acid. Phenylpropanoid glycosides have been isolated from the aerial parts of several *Sideritis* species. Verbascoside (**26**), leucosceptoside (**27**), martynoside (**28**), and lavandulifolioside (**29**) were determined to be the most important compounds with respect to their pharmacological properties (17, 39-42). Verbascoside is a phenolic compound known for its antiproliferative, cytotoxic, antioxidant, and antimetastatic properties.

A variety of biological activities of *Sideritis* species have been reported: anti-inflammatory, anti-ulcer, analgesic, antimicrobial and antifungal, antifeedant, anticataract, immunomodulating, macrophage NOS-2-expression-inhibiting, and hypoglycaemic. Recently, aldose reductase-inhibiting activity, antiproliferative, anticholinesterase and selective oestrogen receptor modulator-like effects have been reported.

Anti-inflammatory activity

The species of the genus *Sideritis* are known in traditional medicine for their anti-inflammatory activity. Numerous studies have validated this therapeutic indication. The diterpenoid contents in several species of *Sideritis* can be correlated with their anti-inflammatory properties.

Hernandez-Pérez and Rabanal (5) studied the anti-inflammatory and analgesic activity of *S. canariensis* var. *pannosa,* and the ethanol extract and the chloroform fraction were both shown to exhibit strong anti-inflammatory and analgesic activities, possibly due to the rich diterpenoid content. The same group also investigated the effects of *S. candicans* Ait. var. *eriocephala* Webb (43). These results showed a significant anti-inflammatory activity against carrageenan-induced paw oedema and against TPA (12-O-tetradecanoylphorbol acetate)-induced ear oedema in mice after oral and topical administration, respectively. Aboutabl et al. (4) postulated that the flavonoids and terpenoids in the dichloromethane fraction of *S. taurica* might be the active anti-inflammatory ingredients.

The lipidic fractions of Sideritis javalambrensis hexane extract exhibit potent anti-inflammatory activity. Namely, in carrageenan-induced paw oedema, oral administration of 50 and 100 mg/kg of the lipidic fraction significantly inhibited the paw oedema formation at different times. In the same way, in TPA-induced ear oedema in mice, the same fraction suppressed ear oedema formation after topical application of 0.25, 0.5 and 1 mg/ear (35, 41 and 53% inhibition, respectively). The lipidic fraction also reduced the histamine release from mast cells and reduced NO generation in macrophages (44). Another study of *S. javalambrensis* extracts revealed that the hexane and methanol extracts possessed the highest anti-inflammatory activ-

Species	*Yield*	*Main constituents*
S. angustifolia Lam.	—	α-Pinene (10.8–20.6%), β -bisabolol (2.5–20.2%), 1,8-cineole (4.6–16.6%)
S. argyrea P. H. Davis	0.45	β-Pinene (19.7%), α-pinene (13.8%)
S. armeniaca Bornm	0.54	β-Pinene (39.3%), α-pinene (16.5%), β-phellandrene (10.5%)
S. bilgerana P. H. Davis	0.26	β-Pinene (51.2%), α-pinene (30.2%)
S. brevidens P. H. Davis	—	β-Pinene (14.1%), epi-cubenol (13.1%), α-pinene (7.9%)
S. caesarea H. Duman, Z. Aytaç & K. H. C. Baser	0.02	β-Caryophyllene (8.3%), caryophyllene oxide (7.4%)
S. chamaedryfolia Cav.	—	Caryophyllene (32.5%), caryophyllene oxide (14.3%)
S. clandestina Hayek ssp. clandestina	0.26	α-Pinene (20.1%)
S. congesta P. H. Davis & Hub.-Mor.	0.83	Muurol-5-en-4-α-ol (11.7%), muurol-5-en-4-β-ol (33.0%)
	0.45	β-Pinene (34.6%), α-pinene (24.6%)
S. curvidens Stapf	0.02	Bicyclogermacrene (20.6%), spathulenol (12.4%)
S. erythrantha Boiss. & Heldr. var. cedretorum	0.70	Myrcene (24.3%), α-pinene (12.4%)
S. erythrantha Boiss. & Heldr. var. erythrantha	0.50	α-Pinene (19.5%), sabinene (10.4%)
S. flavovirens (Rouy) F. Alcaraz, M.Peinado, J. M. Martínez-Parra, J. S. Carrión & P. Sánchez-Gómez	—	Fenchyl acetate (12.0–27.7%), fenchone (11.9–25.3%), α-pinene (8.2–18.7%), 1,8-cineole (1.4–13.4%), limonene (3.4–12.7%)
S. foetens Benth.	—	Thymol (2.3–20.0%), p-cymene (12.3–19.8%), sabinene (8.6–13.4%), α-pinene (5.5–11.6%)
S. hirsuta L.	0.44	β-phellandrene (23.8%), α- phellandrene (9.2%), α -pinene (8.2%)
S. hololeuca Boiss. and Heldr. Apud Bentham	0.02	β -Pinene (35.5%), α-pinene (16.0%), β-phellandrene (9.6%)
S. ibanyezii Pau	0.71	α-Fenchyl acetate (16.0%), sabinene (12.8%), α-pinene (10.7%)
S. lanata L.	0.03	Hexadecanoic acid (10.7%), spathulenol (9.5%)
S. leucantha Cav.	—	α-Pinene (23.6–25.8%), sabinene (7.2–10.4%), fenchone (6.2–10.2%)
S. montana L.	0.16	Germacrene D (41.1%), bicyclogermacrene (10.9%)
S. montana L. ssp. *montana*	0.05	Germacrene D (24.6%), bicyclogermacrene (10.8%)
S. montana L. ssp. *remota*(D'Urv.) P. W. Ball	0.03	Bicyclogermacrene (13.9%), germacrene D (10.3%)
S. mugronensis J. Borja	0.02	δ -Cadinene (2.0– 47.0%), 1,8-cineole (0.4–28.7%), bisabolol (3.0–27.2%), sabinene (0.6–12.6%)
S. ozturkii Aytaç & Aksoy	0.20	α-Pinene (31.1%), β -pinene (20.2%)
S. pauli Pau	0.32	α-Pinene (48.0%)
S. phlomoides Boiss. & Bal.	0.20	β -Caryophyllene (30.7%), α-bisabolol (16.2%)
S. raeseri Boiss. & Heldr. ssp. *attica*	0.37	α-Pinene (24.8%), β -pinene (18.0%)
(Heldr.) Papan & Kokkini	0.17	α-Pinene (28.7%), β -pinene (27.2%)
S. raeseri Boiss. & Heldr. ssp. *raeseri*	0.12	β -Pinene (9.1%)
S. romana L. ssp. *romana*	0.05	Thymol (24.9%), 1-octen-3-ol (12.6%), borneol (9.2%)
S. rubriflora Hub.-Mor.	–	β-Pinene (13.2%), α-pinene (9.9%), epi-cubenol (7.8%)
	0.13	β-Caryophyllene (18.8%), nerolidol (12.1%)
S. scardica Griseb.	0.03	β-Pinene (17.9%), carvacrol (14.8%), α-pinene (7.3%)
	0.40	Menthol (8.5%), 9-eicosene (6.3%), geraniol (5.6%)
S. sipylea Boiss.	—	Verbenone (15.2%), terpineol (9.5%) / carvacrol (81.2%), terpinen-4-ol (8.2%)
S. stricta Boiss. et Heldr.	0.40	α-Pinene (35.2%)
Apud Bentham	0.63	β-Pinene (30.0%), α-pinene (12.9%)
S. syriaca L. Leaves	0.05	Hexadecanoic acid (31.1%), epi-α-bisabolol (14.5%)
Inflorescences	0.07	*epi*-α-Bisabolol (25.7%), benzyl benzoate (17.7%)
	0.12	Myrcene (50.5%)
S. syriaca L.	—	α-Pinene (19.5%), carvacrol (11.9%), thymol (7.2%)
S. syriaca L. ssp. *syriaca*	0.19	Carvacrol (33.7%)
S. taurica Stephan ex Willd.	0.08	α-Bisabolol (10.3%), β-pinene (9.3%)
S. tmolea P. H. Davis	0.33	α-Cadinol (21.9%), β-caryophyllene (10.6%)
	0.30	α-Bisabolol (8.4%)
S. tragoriganum Lag.	—	α-Pinene (7.8–17.7%), 1,8-cineole (6.8–15.9%), β -caryophyllene (0.3–14.6%), caryophyllene oxide (10.2%), fenchone (6.1–7.8%)
S. tragoriganum × *S. leucantha*	—	α-Pinene (50.1%), sabinene (10.6%)
S. vulcanica Hub.-Mor.	0.02	β -Caryophyllene (10.2%), hexadecanoic acid (9.7%)
S. vuralii H. Duman & K. H. C. Baser	0.10	β -Pinene (35.3%), 1,8-cineole (14.6%), α-pinene (14.5%)

ity against adjuvant-carrageenan-induced inflammation in the chronic stage, and no effect was observed in the acute phase (9). The hexane extract of this *Sideritis* species also showed a strong anti-inflammatory effect in a croton oil-induced corneal oedema model in rabbits during the chronic stage (45). Later, several novel labdane-type diterpene derivatives were reported as active anti-inflammatory constituents of the n-hexane extract of *S. javalambrensis*. Based on *in vitro* studies, it has been concluded that these compounds interact with the eicosanoid system, possibly inhibition ofby inhibiting the phospholipase A2 enzyme.

Apart from the flavonoid and diterpene derivatives, the sterol fractions of the *Sideritis* species were also shown to possess anti-inflammatory and immunomodulating activity. The lipid fraction from *S. javalambrensis* (44) and a sterol fraction composed of campesterol, stigmasterol and β-sitosterol from *S. foetens* (8) were also reported as active components. The oral administration of 30 and 60 mg/kg of the sterol fraction inhibited the oedema formation in the mouse paw between 3 and 7 h after carrageenan administration. The highest inhibitory effect was obtained after 3 h, with values of 30.1% for 30 mg/kg and 37.4% for 60 mg/kg of the sterol fraction. In addition, the topical application of 0.25, 0.5 and 1 mg/ear of the sterol fraction reduced the oedema formation induced by TPA in mice with inhibition percentages of 41, 43 and 58.7%, respectively, associated with a reduction in the neutrophil infiltration into inflamed tissues.

As anti-inflammatory constituents, the polymethoxyflavone isolated from *S. tragoriganum*, 5-*O*-demethylnobiletin, may act through the direct inhibition of 5-LOX, without affecting the expression of COX-2. It is known that lipid peroxides promote arachidonic acid metabolism and that a redox agent, such as phenolic derivatives, can inhibit the oxidation of arachidonic acid by 5-lipoxygenases; thus, antioxidant and free radical-scavenging flavonoids could prevent the generation of inflammatory mediators. The anti-inflammatory and antinociceptive activities of *S. ozturkii* extracts were confirmed, and a flavone glycoside, ozturkoside C, was isolated as one of the active ingredients. Despite a high number of studies reporting the anti-inflammatory and antinociceptive activities of several *Sideritis* species, only two flavonoids have been isolated and defined as the active constituents, hypolaetin-8-glucoside and 5-*O*-demethylnobiletin. Hypolaetin-8-glucoside shows a chemical structure very close to that of ozturkoside C, both having luteoline-type flavone glycoside structure (3). Another anti-inflammatory labdane derivative, andalusol, was also isolated from the acetone extract of *S. foetens,* and its activity profile was elucidated (9). Andalusol exerted *in vivo* anti-inflammatory activity when tested in different inflammation models in mice (carrageenan-induced paw oedema and TPA-induced ear oedema). Oral administration of andalusol inhibited oedema formation, especially the late phase of paw inflammation (5 ± 7 h after carrageenan injection). Andalusol also exerted topical anti-inflammatory activity 4 h after TPA ear application, inhibiting oedema formation and cell infiltration. This compound affected various leukocyte functions and decreased the histamine release from the mast cells. The activation of macrophages with pro-inflammatory cytokines and bacterial cell wall components promotes the synthesis and release of large amounts of nitric oxide (NO), eicosanoids and bioactive lipids, such as prostaglandins and leukotrienes, mediators involved in the inflammatory onset. Recently, inhibitors of inducible nitric oxide synthase, the isoenzyme responsible for the high-output NO synthesis, have been proposed as anti-inflammatory agents, mainly because the inhibition of exacerbated NO formation may be of therapeutic benefit in these disorders. Moreover, a role of andalusol's effect on the macrophage expression of NOS-2 has been reported in *in vivo* inflammation models, such as carrageenan-induced hind paw inflammation in the rat. In addition, the action of andalusol on NF-kB activity has been evaluated, but the experiments carried out after simultaneous stimulation with LPS and IFN-g strongly suggested that in addition to NF-kB, it is likely that there is an inhibitory action on the IFN-g signalling. Indeed, this phenomenon has been described for triterpenes, and if it is also the case for andalusol, it opens additional perspectives for the study of the therapeutic action of these molecules (9).

In addition, anti-inflammatory activity studies have been conducted using isolated flavonoids. The study of the anti-inflammatory activity of a series of glycosides/aglycone pairs, through the inhibition of eicosanoid generation via the 5-lipoxygenase and cyclo-oxygenase pathways in elicited rat peritoneal leukocytes stimulated with calcium ionophore, revealed the structural-activity relationship. Among these pairs, hypolaetin-8-glucoside and its corresponding aglycone, hypolaetin, which was isolated from *S. mugronensis*, ,were studied. The results showed that hypolaetin inhibits the 5-lipoxygenase enzyme activity in a more powerful and selective way than hypolaetin-8-glucoside (IC50=4.5μM, IC50=56μM, respectively). If we compare these results with others obtained from other pairs, it is confirmed that the sugar moiety reduces the inhibitory potency. In the same study, a structural–activity relationship in flavonoids was found: those flavonoids with a catechol group in the B ring are potent and selective inhibitors of 5-lipoxygenase. However, flavonoids with hydroxyl substituents in their structures, except for the B-ring, are selective against the cyclo-oxygenase enzyme (46).

Antioxidant activity

In all aerobic organisms, including human beings, the production of reactive oxygen species (**ROS**) is balanced by an antioxidant defence system. ROS in the forms of superoxide anion, hydroxyl radicals and hydrogen peroxide, which are generated by normal metabolic processes or from exogenous factors and agents, affects DNA, proteins and most biological molecules containing a lipid component of polyunsaturated fatty acids. A serious imbalance between the production of ROS and the antioxidant defence system is responsible for oxidative stress. Thus, ROS play

Table 1. Main constituents and yields of the essential oils of *Sideritis spp.*, as previously reported.

an important role in the aetiology of many diseases and of ageing. Antioxidant defence systems that prevent the oxidative damage by ROS consist of flavonoids, carotenoids, phenolic compounds, vitamins and antioxidant enzymes. The role of antioxidants has attracted much interest with regard to their protective effect against free radical damage, which may be the cause of many diseases, including cancer. The results have shown that the antioxidant activities of the extracts do not necessarily correlate with high amounts of phenolics. Although the extracts were found to be effective natural antioxidants, their potential exploitable beneficial effects and their safety in humans need to be proven in clinical trials.

The antioxidant activity was evaluated for methanolic extracts (0.02% and 1%) from twenty-seven *Sideritis* species by measuring Fe2+-induced linoleic acid peroxidation. The antioxidant activity improved with increasing concentration in every case. Moreover, studying the free radical scavenging activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method showed that the higher total phenolic content, the higher the free radical scavenging activity: *S. amasiaca and S. germanicopolitana* ssp. *viridis*, with the highest amounts of phenolic derivates, were the most active (47).

The total antioxidant capacity of *S. sipylea* was determined by the thiocyanite method. The obtained extracts in the linoleic acid emulsion were able to reduce the formation of peroxides. *S. sipylea*, an endemic species in Turkey, showed radical scavenging antioxidant activities (48). *S. syriaca* ssp. *syriaca*, endemic in the mountainous regions of Crete (Greece) and widely used for the preparation of traditional infusions, was proved to possess good antioxidant capacity in its more polar (diethyl ether, ethyl acetate and butanol) extracts. The phytochemical analysis of the extracts revealed the presence of significant quantities of phenylpropanoid acid derivatives and flavonoids (mainly flavones) (14, 49), which have been considered responsible for antioxidant activity. The ethyl acetate fraction possessed the highest antioxidant activity, which could be attributed to the presence of phenolic compounds, such as apigenin and isoscutellarein glycosides.

This relation between the antioxidant activity and the phenolic content has also been found for the methanolic extracts from *S. ozturkii* and *S. caesarea*, and the results obtained from the DPPH method were 41.68±1.96% and 72.47±0.73%, respectively, at 100 ppm concentration. The higher radical scavenging activity is shown by higher percentage values of inhibition. The total phenolics and total flavonol content were higher for the latter species (50). The present study suggests that the extracts of these plants are a potential source of natural antioxidant agents.

The antioxidant potential was investigated for the *ent*-kauranes and the petroleum ether and acetone extracts of the aerial parts of *S. arguta* by three methods, *β*-carotene bleaching, free-radical scavenging and superoxide-anion scavenging activity. Both the methanol and the acetone extracts exhibited similar antioxidant activity in every assay. The petroleum ether extract showed antioxidant ability by the β-carotene bleaching and superoxide-anion methods. However, it did not show any antioxidant ability by the DPPH method. The only active diterpenoid was 7-epicandicandiol.

The methanolic, ethereal, butanolic and aqueous extracts from the aerial parts of *S. perfoliata* subsp. *perfoliata* and the isolated flavonoids and phenylpropanoid glycosides from this species were evaluated for their antioxidant properties in different *in vitro* assays, including scavenging DPPH and TBA (thiobarbituric acid) lipid peroxidation (2).

Moreover, the antioxidant activities of *S. javalambrensis* and *Sideritis libanotica subsp. linearis* were reported, with phenylpropanoid glycoside acteoside recognised as a reducing agent able to interact with free radical species (of relevance to the autoxidation mechanism) (2, 19, 47).

In Greece, *Sideritis* species have been used as flavouring additives and preservatives in olive oil. Because various flavonoids have been identified in *Sideritis* species and because it is known that these have high antioxidant activity, it is possible that *S. euboea* can be used as a source of natural food antioxidants with economic benefits, especially for Greece (51). Despite the traditional use of this plant, in a placebo-controlled clinical trial recently performed to evaluate its antioxidant activity, no significant differences were found in the blood biochemical parameters (glutathione, nitrites, coenzyme Q10 or vitamins C, A and E) between the placebo and the intervention groups.

The antioxidant activity of *S. raeseri* Boiss. et Heldr. subsp. *raeseri* methanolic extract, evaluated using Co(II)/EDTA-induced luminol chemiluminescence and the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical assay, was found to be moderate. The activity may be related to the presence of 5- and 8-*O*-disubstituted flavones. The obtained results were IC50 1.63 mg mixture/mg DPPH and EC50 8.3 μg/mL, respectively; these activities are moderate when compared with the potent antioxidants quercetin and trolox (26). All the compounds detected in the methanolic subfraction of *S. raeseri* investigated are 7-*o*-(β-allopyranosyl-(1→2)-β-d-glucopyranosyl derivatives of 5,8-dihydroxyflavones with different substitutions in the B-ring. Four of these compounds are monoacetylated at the C-6‴ of the allose moiety and one at the C-6″ of the glucose moiety. An important antioxidant activity would have been expected for these compounds as it was reported that 7,8-dihydroxyflavone showed antioxidant activity similar to that of quercetin, although it lacked any substitution on the B-ring and at the 3-position. In 4′,5,8-trihydroxy-6,7-dimethoxy flavone, the enhanced antioxidant activity was probably due to the hydroxy and two methoxy groups in ring A. Thus, it is obvious that for flavones with 5,8-dihydroxyl substitution in ring A and in some cases with a free hydroxyl group at C4′, a more significant antioxidant activity would be expected. Although *p*-hydroxy phenols (ring A) are oxidant systems, a moderate activity has been observed for these compounds. It is probably explained by the fact that in 5,8-dihydroxy flavones, the reducing capacity of the *p*-hydroxy system is suppressed by (1) the intra-

molecular hydrogen bond between the OH in C-5 and the carbonyl group and (2) the enhanced inductive effect of the 7-*o*-glycoside substituent, as opposed to the free hydroxyl group. Furthermore, a systematic comparative study of the antioxidant activity between flavones 5-OH, 7- glycosides and 7-glycosides lacking OH- substitution on C-5 with or without an 8-OH group is in our future plans.

Anti-ulcerogenic activity

Plants from the genus *Sideritis* have also long been used in traditional medicine for their gastroprotective properties. These plants provide a source of natural products with anti-ulcerogenic action, proved in animal tests *in vivo* and *in vitro,* including, among other flavonoids, hypolaetin-8 -glucoside. These flavonoids reduced gastric lesions and drug-induced ulcers in rats by increasing mucus production and decreasing the gastric acidity. According to other studies (52), the presence of a pyrocatechol group at the 3'–4' position in the flavonoid skeleton is related to a higher anti-ulcerative activity.

The aerial parts of *S. incana* var. *virgata, S. funkiana* ssp. *funkiana, S. funkiana* ssp. *talaverana* and *S. hirsuta* were prepared as decoctions and orally administered to rats suffering from indomethacin and stress-induced ulcers. This *in vivo* study of anti-ulcerative activity demonstrated that *S. incana* var. *virgata, S. funkiana* ssp. *funkiana, and S. funkiana* ssp. *talaverana* were more active against indomethacin-induced ulcers, whereas *Sideritis hirsuta* was more effective against stress-induced ulcers. *S. caesareae* was confirmed to possess strong biological activity against ethanol-induced gastric ulceration in rats. Also, studies have been performed on the anti-ulcerogenic activity of hypolaetin-8-O-β-d-glucoside, a flavonoid isolated from *S. leucantha* and present in several *Sideritis* species (*S. mugronensis*, *S. angustifolia* and *S. saetabensis*) (52-54).

In addition, a strong dose-dependant activity against *Helicobacter pylori* was observed for *S. italica* essential oil, in concentrations between 5 and 25 μg/mL (1).

Analgesic activity

A *p*-benzoquinone (PBQ)-induced abdominal constriction test was performed on mice for the determination of antinociceptive activity in *S. brevibracteata* (10). The results are in good accordance with the uses of this genus. Namely, the dried flowering spikes of the *Sideritis* species are used as herbal tea in the western and southern coastal regions of Turkey due to their pleasant aroma. The n-butanol extract of *S. brevibracteata* exhibited the highest antinociceptive activity. It was considered that the active compounds for antinociceptive activity could be flavonoids, which are the major components of the n-butanol fraction (3). The anti-nociceptive effects of the ethanol extract, as well as the aqueous and chloroform fractions from *S. lotsyi* var. *mascaensis*, have been investigated using the writhing test induced by acetic acid in mice. At 250 mg/kg p.o., the ethanol extract significantly inhibited the writhing responses at different times and was more active than the other evaluated fractions. The chloroform fraction was more active than the aqueous extract at 125 mg/kg p.o (5). A dose of 400 mg/kg p.o. of petroleum ether extract obtained from the flowering aerial parts of *S. taurica* exhibited an analgesic activity similar to that produced by a doseage of 400 mg/kg of acetylsalicylic acid (4). The antinociceptive activities of *S. ozturkii* extracts were confirmed, and a flavone glycoside, ozturkoside C, was isolated as one of the active ingredients, as already mentioned (3).

Antiproliferative activity

Demirtas et al. (2009) reported that the methanolic extract from the aerial parts of *S. libanotica* ssp. *linearis* showed a significant antiproliferative activity against three human cell lines, Vero cells (African green monkey kidney), C6 cells (rat brain tumour cells) and HeLa cells (human uterus carcinoma) (11).

Anti-HIV activity

An *in vitro* study was carried out on H9 lymphocyte cells to determinate the anti-HIV activity of linearol and of twenty-six semisynthetic ent-kaurene derivatives from linearol. The results showed that linearol was inactive (55).

Anticholinesterase activity

The petroleum ether and acetone extracts of the whole plant of *Sideritis congesta* P.H. Davis & Hub.-Mor. and the isolated compounds belonging to ent-kaurane diterpenoids (epoxyisolinearol, sideroxol, sideridiol, siderol, 7-epicandicandiol, linearol and sidol) were evaluated for their anticholinesterase activity, and most of the diterpenes exhibited weak acetylcholinesterase inhibitory activity. However, almost all diterpenes exhibited some inhibitory activity against butyrylcholinesterase; in particular, sideroxol and 7-epicandicandiol exhibited better BChE inhibitory activity than the standard compound galanthamine (56).

Selective oestrogen receptor modulator (SERM) activity

Traditional therapeutic agents (selective oestrogen receptor modulators or SERMs, biphosphonates, calcitonin) may have serious side effects or contraindications. In an attempt to find food components with the potential to act as SERMs, plant aqueous extracts derived from the Greek flora *S. euboea* and *S. clandestina* were submitted in a series of *in vitro* biological assays reflective of the SERM profile. Their ability (a) to stimulate the differentiation and mineralisation of osteoblastic cell culture by histochemical staining for alkaline phosphatase and Alizarin Red-S staining, (b) to induce, like antioestrogens, the insulin growth factor binding protein 3 (IGFBP3) in MCF-7 breast cancer cells, and (c) to proliferate cervical adenocarcinoma (HeLa) cells were examined using the MTT assay . The data revealed that all the plant extracts studied at a concentration range of 10-100 μg /mL stimulate osteoblastic cell differentiation and exhibit an antioestrogenic effect on breast cancer cells without proliferative effects on cervical adenocarcinoma

cells. The presence of estradiol inhibited the antioestrogenic effect induced by the extracts in MCF-7 cells, suggesting an oestrogen receptor-related mechanism (13).

Antimicrobial activity

There are several reports on the antimicrobial activity of *Sideritis* essential oil. The antimicrobial activity of the essential oils of *S. perfoliata* and *S. trojana* was tested against *Escherichia coli* (NRRL B-3008), methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterobacter aerogenes* (NRRL 3567), *Salmonella typhimurium* (NRRL B-4420), *Bacillus cereus* (NRRL B-3711), *Staphylococcus epidermidis* (ATCC 12228) and *Candida albicans*. The results of the antimicrobial assays indicated that *E. coli*, methicillin-resistant *S. aureus* (MRSA), *E. aerogenes*, *B. cereus,* and *C. albicans* were moderately inhibited by the oil of *S. trojana* with MIC values of 125 to 250 mg/mL, which were lower than the MIC values of the standard antimicrobial agent. The oil showed a strong inhibitory effect against *S. epidermidis* with a MIC value of 62.5 mg/mL. Except for *C. albicans*, *S. perfoliata* oil, however, was less active (125 to 500 mg/mL) against the test microorganisms. The occurrence of a higher content of oxygenated derivatives of mono and sesquiterpenes (20%) in the oil of *S. trojana* may be responsible for the better antimicrobial activity (35, 57).

In addition, there are several reports about the antimicrobial activity of essential oil from Spanish *Sideritis* species. *S. angustifolia, S. funkiana, S. javalambrensis, S. leucantha, S. mugronensis* and *S. tragoriganum* inhibited the growth of Gram-positive bacteria, *Staphylococcus aureus, Mycobacterium phlei* and the fungi *Candida albicans*, whereas they did not show any activity against Gram-negative bacteria. Similar results were obtained in the investigation of the essential oils of *S. curvidens* and *S. lanata*, which had no effect against any Gram-negative bacteria, but they showed significant activity against Gram-positive bacteria (58, 59).

In contrast, essential oils from *S. cilicica* and *S. bilgerana* exerted a significant inhibitory effect against several Gram-negative (*Salmonella typhimurium*, *Escherichia coli*) and Gram-positive (*Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermidis*) bacteria, with a MIC value from 0.125 to 0.5 mg/mL, as well as against *Candida albicans* (MIC 0.03 mg/mL). This antibacterial activity could be due to the presence of α-pinene and β-pinene as the main constituents of both species (60). Also, *S. italica* essential oil was investigated because of its antimicrobial activity, which has been shown to be higher against Gram-negative than Gram-positive bacteria, especially against *Pseudomonas aeruginosa* (1).

Not only the essential oils but also the various *Sideritis* extracts possess significant antibacterial activity. According to the study performed by Sagdic et al. (50), the methanolic extracts of *S. ozturkii* and *S. caesarea* had considerable antimicrobial activity. The fifteen microorganisms used as test organisms were *Aeromonas hydrophila* ATCC 7965, *Bacillus brevis* FMC 3, *B. cereus* FMC 19, *B. subtilis* ATCC 6630, *B. subtilis* var. *niger* ATCC 10, *E. coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Morgenella morganii*, *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 28213, *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223 and *Saccharomyces cerevisiae* BC 5461. Linearol, foliol, epicandicandiol and siderol, which are found in the mentioned *Sideritis* species, were also investigated for antibacterial activities, and epicandicandiol had the highest antimicrobial activity against *E. coli*.

The acetone and methanol extracts of *Sideritis tmolea* P. H. Davis were tested against standard bacterial strains, such as *Escherichia coli, Staphylococcus aureus, Mycobacterium smegmatis, Mycobacterium tuberculosis* H37Ra (ATCC 25177) and the yeast *Candida albicans*. The results of the activity studies showed no significant antimicrobial or antituberculous activity for the *Sideritis* species' crude acetone and methanol extracts (61).

CONCLUSIONS

The genus *Sideritis* provides a wide range of research possibilities. This work is a comprehensive overview of the botanical, phytochemical and pharmacological aspects of the genus *Sideritis*, objectively presenting the scientific basis of its ethnopharmacological use.

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NEUROLOGICAL SIGNS AND SYMPTOMS IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE

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NEUROLOŠKI SIMPTOMI I ZNACI KOD PACIJENATA SA AUTOIMUNSKOM BOLEŠĆU ŠTITASTE ŽLEZDE

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ABSTRACT

Neurological and/or psychiatric signs and symptoms can characterizise the clinical picture of encephalopathy associated with autoimmune thyroid diseases and high levels of serum antithyroid autoantibodies. To the best of our knowledge, the literature does not include data on neurological abnormalities in patients with autoimmune thyroid diseases without encephalopathy. Therefore, the aim of this study was to analyse the neurological signs and symptoms that are not associated with a previously identified disease in patients with autoimmune thyroid diseases. This study included 66 patients who were diagnosed with autoimmune thyroid disease. Before the neurological examination, a detailed history of neurological symptoms was obtained for each patient. No neurological symptoms had been present before the test in 47 of 66 patients (71%). Of the remaining 19 patients (29%), 13 of 66 (20%) patients had headache. Among patients with headache, the concentrations of thyroid peroxidise antibodies were slightly higher than in patients without headache, though the difference was not statistically significant (p=0.380, Mann-Whitney test). The patients who took part in this study complained of other neurological symptoms including vertigo (two patients, 3.0%), tingling of hands (two patients, 3.0%), transient weakness of one leg (one patient, 1.5%) and forgetfulness (one patient, 1.5%). Electroencephalography was performed only in patients with neurological symptoms and was normal in all of these patients. Hashimoto encephalopathy is probably not as rare as predicted, but, among our patients with autoimmune thyroid disease, we did not recognise any patients meeting the required diagnostic criteria for encephalopathy. Some of our patients had headache, which was not linked with any previously identified disease.

Keywords: *autoimmune thyroid disease, encephalopathy, thyroid peroxidise, antibodies, headache*

SAŽETAK

Simptomi i znaci neuroloških i/ili psihijatrijskih bolesti (ili: Neurološki i/ili psihijatrijski simptomi) čine kliničku sliku encefalopatije koja je povezana sa autoimunskom bolešću štitaste žlezde i visokom koncentracijom atitireoidnih autoantitela u serumu obolelih. Prema našem saznanju, nema podataka o tome da li postoje diskretni neutrološki poremećaji kod pacijenata sa autominskom bolešću štitaste žlezde bez prethodno dijagnostifikovane encefalopatije.

Cilj našeg rada je da se ispita da li kod obolelih od autoimunske bolesti štitaste žlezde postoje neurološki simptomi i znaci koji se ne mogu povezati sa (objasniti) ranije poznatim bolestima. U ispitivanje je uključeno 66 pacijenata sa dijagnostifikovanom autoimunskom bolešću štitaste žlezde. Pre pregleda uzeta je detaljna anamneza o simptomima koji mogu biti prouzrokovani neurološkim bolestima ispitanika. Bez neuroloških simptoma bilo je 47 (71%) pacijenata. Od preostalih 19/66 (29%) pacijenata, 13/66 (20%) pacijenata imalo je glavobolju. U podgrupi pacijenata sa glavoboljom, koncentracija antitela specifičnih za tireoidnu peroksidazu bila je nešto veća nego kod pacijenata bez glavobolje, ali razlika nije bila statistički značajna (p=0.380, Mann-Whitney test). Od drugih simptoma, pacijenti uključeni u ovo ispitivanje žalili su se na vrtoglavicu (dva pacijenta, 3.0%), trnjenje ruku (dva pacijenta, 3.0%), prolaznu slabost jedne noge (jedan pacijent, 1.5%) i zaboravnost (jedan pacijent,1.5%). Elektroencefalografija je uradjena samo kod pacijenata sa neurološkim simptomima i nalazi su bili normalni kod svih ispitanika. Hashimoto encefalopatija je verovatno češća nego što se pretpostavlja, ali kod naših pacijenata sa autoimunskom bolešću štitaste žlezde nije nadjen nijedan pacijent koji bi zadovoljio potrebne dijagnostičke kriterijume. Deo naših pacijenata imao je glavobolju, koja se ne može povezati sa ranije poznatim bolestima.

Ključne reči: *autoimunska bolest štitaste žlezde, encefalopatija, tireoidna peroksidaza, antitela, glavobolja*

Abbreviations: Abs - autoantibodies, **AITD** - Autoimmune thyroid diseases , **fT4** - free thyroxine , **GD** - Graves' disease, **HE** - Hashimoto encephalopathy, **HT** - Hashimoto's thyroiditis, **TPO** - thyroid peroxidise , **TSH** - thyroid stimulating hormone

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INTRODUCTION

Autoimmune thyroid diseases (AITD) are complex diseases that have cellular and humeral immune responses targeted at the thyroid gland (1, 2, 3). AITD include **Hashimoto's thyroiditis** (HT) and Graves' disease (GD), both of which involve the infiltration of the thyroid by T and B cells that are reactive with thyroid antigens and production of antithyroid autoantibodies (4, 5, 6, 7). Encephalopathy associated with autoimmune thyroid diseases and high levels of serum antithyroid antibodies was first described in 1966 (8, 9, 10) and was named Hashimoto encephalopathy (HE). Since then, more than one hundred cases have been reported, demonstrating a direct link between AITD and the development of encephalopathy (11, 12). The prevalence of HE is 2.1/100,000 (13). Neurological and/or psychiatric signs and symptoms are constituents of the clinical picture of HE. The clinical features can be quite variable. The most frequent clinical manifestations include stroke-like episodes and a constellation of signs that mimics Creutzfeldt-Jakob disease. The most frequent signs are epileptic seizures, subacute confusion, myoclonus, cognitive impairment or dementia, fluctuations in consciousness, ataxia, tremor, personality disturbances, vertigo, headache, etc. (14). Although rare, HE may be an under-recognised condition because its clinical presentation overlaps with common neurological and psychiatric disorders.

To the best of our knowledge, there isare no data in the literature on neurological abnormalities in patients with AITD without encephalopathy. For that reason, the aim of this study was to analyse the neurological signs and symptoms in patients with AITD that cannot be associated with any previously identified disease.

MATERIAL AND METHODS

This study was conducted from October-December of 2009 at the Centre of Nuclear Medicine and the Department of Neurology, Clinical Centre Kragujevac, and it was approved by the Ethics Committee of the Clinical Centre Kragujevac.

The study included 66 patients. All blood samples were originally obtained for diagnostic purposes. Blood samples (10 ml) were obtained from each patient and the serum was separated by centrifugation at 2000 rpm for 15 minutes. The sera were frozen at -20°C for storage and then were thawed and assayed. After increased concentrations of thyroid peroxidise autoantibodies (TPO Abs) were found, the patients were informed about the study protocol and were included in the study for further testing. Additionally, concentrations of free thyroxine (fT4) and thyroid stimulating hormone (TSH) were measured from the collected sera.

The concentration of TPO Abs was measured using a radioligand assay (TPO-Ab-CT, *Cis-Biointernational*, France) according to the manufacturer's instructions. The lower detection limit for this assay was 8U/ml. The cut off value of autoantibodies specific for thyroid peroxidase was determined based on the manufacturer's recommendations. The measured TPO Ab values were analysed based on a value of 130 U/ml; autoantibody concentrations higher than 130 U/mL were considered "positive".

The concentration of free thyroxine was measured using a radioimmunoassay (*Cis-Biointernational*, France). The detection limit for this assay was 0.5 pg/ml, with a reference range of 7-18 pg/ml.

The concentration of thyroid stimulating hormone was measured using an immunoradiometric assay (IRMA TSH, Zemun, Serbia) with a detection limit of 0.056 mIU/L and a reference range 0.3-5.5 mIU/L.

A detailed history of symptoms that could be associated with neurological diseases was obtained before the neurological examination. The patient answered questions about his or her symptoms, which may have resembled a cerebrovascular accident, Creutzfeldt-Jakob disease, dementia, epilepsy, myelopathy, damage to the peripheral nervous system and cerebellar syndrome (15). We did not examine mental status. Any symptoms potentially associated with HE were carefully recorded. The neurological examination assessed the cranial nerves, the central and peripheral nervous system and cognitive function. We assessed cognitive function with the mini-mental status exam (MMSE). Impaired cognitive function indicative of dementia (MMSE score of less than 24) was not detected in any of our patients. Two independent neurologists performed the neurological examination on all of the patients. We used a set of clinical criteria for the diagnosis of definite, probable or possible HE, established by Tamagno et al. (16). Mental function was not examined.

Electroencephalography (EEG) was performed only in patients with neurological symptoms. EEG was recorded with a Nihon Kohden 9200J/K apparatus with 25 electrodes placed in accordance with the 10/20 system.

The results obtained were analysed using descriptive statistical methods and the Mann-Whitney test (rank sum test). The tests were conducted using SPSS 10.0 and the MS EXCEL programme.

RESULTS

This study included 66 patients diagnosed with autoimmune thyroid disease, including 64 women (97%) and 2 men (3%). The mean age was 50 years and the SD was 12.19 years. The youngest patient was 22 years old while the age of the oldest patient was 75 years. The distribution of patients by age is shown in Figure 1.

Figure 1.

All patients had increased levels of TPO antibodies. The average value of TPO Ab was 4,176 U/mL (minimal concentration 280 U/ml, maximum 12125 U/ml), with a SD of 2,887 U/mL. During testing, 52 subjects (9%) had normal thyroid function, 6 (9%) had subclinical hypothyroidism, 3 (4%) had overt hypothyroidism, 2 (3%) had subclinical hyperthyroidism and 3 (5%) had overt hyperthyroidism (Figure 2).

Figure 2.

Of the 66 patients, 47 (71%) had no neurological symptoms before the test. Of the remaining 19 patients (29%), 13/66 patients (20% of the total) had headache. One patient had been diagnosed with temporal arteritis, and the other patients had no known neurological or vascular disorders that occur with headache. In the patients with headache, the concentrations of TPO Abs were slightly higher than in the patients without headache, but the difference was not statistically significant (p=0.380, Mann-Whitney test). Patients who took part in this study also complained of other neurological symptoms. Two patients (3%), one of whom had diabetes mellitus (DM), complained of vertigo. Two patients (3%), one of whom had arterial hypertension, also complained of tingling of the hands. One patient (1.5%) complained of transient weakness of one leg, and one patient (1.5%) who also had Raynaud's disease and Sjogren's syndrome complained of forgetfulness. The distribution of neurological symptoms is shown in Figure 3. The EEG was normal in all these patients.

Figure 3.

We found several patients with symptoms of suspected HE: 71% of patients had no symptoms, 20% had headaches, 3% had vertigo, 3% had tingling of the hands, 1.5% had transient weakness of the legs and 1.5% had forgetfulness. The patient with forgetfulness had an MMSE score greater than 24. Of these patients, none met the required diagnostic criteria for HE.

In our group of patients, 10 of 66 (15.1%) had been treated for neurological diseases. Of these, 4 of 66 (6.1%) had polyneuropathy (of these, 3 had DM). Brain infarct had been diagnosed in 4 of 65 (6.1%). One patient had been diagnosed with a transient ischemic attack of the posterior circulation, and another patient had been diagnosed with multiple sclerosis. It is interesting to note that, among the patients with previous brain infarction, one had hypertension while another patient had diseases of the connective tissue (Sjogren's syndrome and Raynaud's disease), while the remaining two (one of whom was 47 years old at the time of brain infarction) had not had any known systemic or vascular diseases.

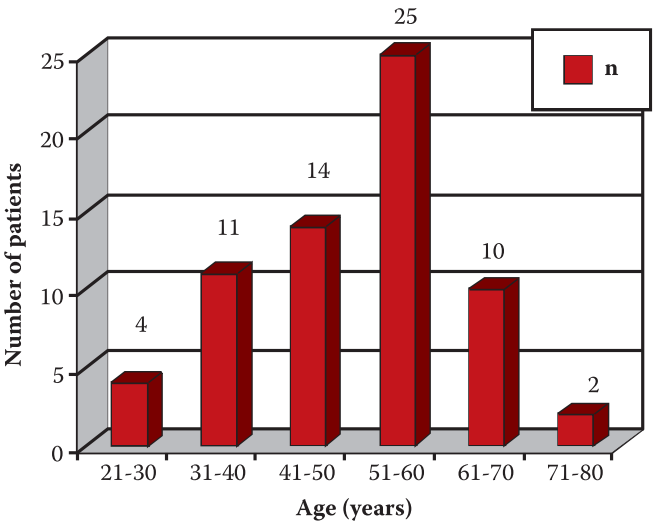

Figure 1. Age of patients with autoimmune thyroid disease.

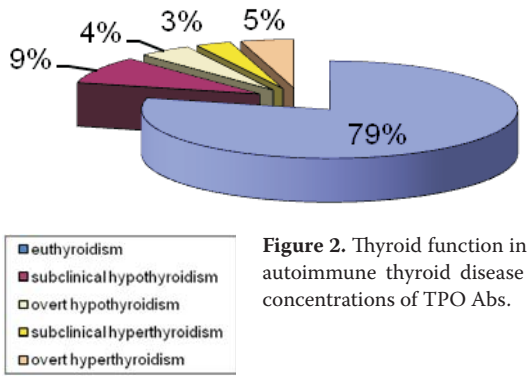

Figure 2. Thyroid function in patients with autoimmune thyroid disease and elevated concentrations of TPO Abs.

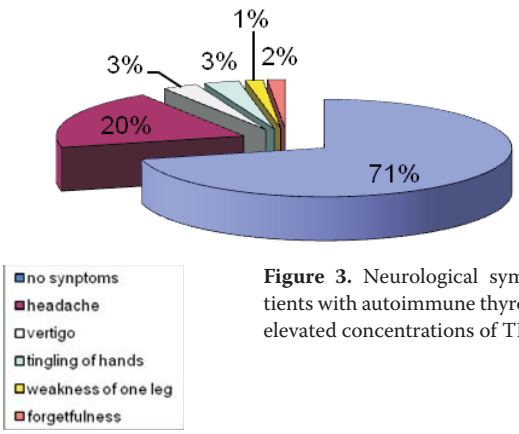

Figure 3. Neurological symptoms in patients with autoimmune thyroid disease and elevated concentrations of TPO Abs

DISCUSSION

The neurological signs and symptoms of 66 patients with autoimmune thyroid disease are presented in this paper. The AITD was confirmed by increased concentrations of TPO Abs in patients' sera.

The most common symptom among our patients was headache, which was found in 20% of the subjects. The other symptoms were present to a significantly lesser extent. Among all of the patients but one who experienced headache, there were no previously diagnosed diseases that were contributory. Headache as a symptom of posterior reversible encephalopathy syndrome have already been presented in patients with AITD (17, 18) and in other autoimmune diseases, especially systemic lupus erythematosus (19, 20).

Given that there are no data on the frequency of headache in patients without TPO Abs, the frequency of headache found in our patients could be compared only with data from the literature the frequency of head-

ache in general population. According to the published data (21, 22), the frequency of headache varies from 8.4% to 30% and depends on many factors that were not examined in our study. Because the percentage of our patients with headache fits the frequency of headache in the general population, we cannot conclude that the headaches in our patients with AITD areis connected with the anti-thyroid autoimmune process. However, because the causes of headache are numerous and have not been thoroughly researched, the possibility that the headache experienced by some of our patients is connected with AITD cannot be excluded.

In patients with headache, the concentrations of TPO Abs were slightly higher than in patients without headache, but the difference was not statistically significant. To the best of our knowledge, there are no data in the literature about the connection between thyroid peroxidase autoantibodies and headache. Although high titres of anti-TPO antibodies were found in almost 100% of cases of reported Hashimoto encephalopathy, the anti-TPO antibody titre did not correlate with the severity of the disease (23).

Other neurological signs and symptoms were found in one or two patients, and the majority of these could be related to a neurological disease that had been previously diagnosed. For this reason, we will not pay particular attention to those symptoms in this discussion.

HE is probably not as rare as predicted. However, among our patients with autoimmune thyroid disease, we did not recognise any patients thatwho met diagnostic criteria for HE. Some of our patients suffered from headache that was not related to any previously identified disease.

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COMPLETE Y-SHAPED THROMBUS REMOVAL WITH A SIMPLE QUICKCAT THROMBECTOMY DEVICE IN PATIENTS WITH SUBACUTE STEMI AND INFLAMATORY BOWEL DISEASE

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POTPUNA EKSTRAKCIJA "Y" TROMBA JEDNOSTAVNIM SISTEMOM ZA TROMBEKTOMIJU KOD PACIJENTA SA SUBAKUTNIM INFARKTOM MIOKARDA I ZAPALJENSKIM OBOLJENJEM CREVA

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ABSTRACT:

Despite the controversial and inconsistent data in the literature, thrombectomy aspiration devices are increasingly used in everyday clinical practice. We present an interesting case report of an acute coronary syndrome patient who suffered from chronic inflammatory bowel disease. During diagnostic colonoscopy in a regional hospital, she developed ST elevation acute myocardial infarction (STEMI) with atrial fibrillation and heart failure. Treating this particular subpopulation of patients with such comorbidities presents challenges.

Key words: *acute myocardial infarction, aspiration thrombectomy*

SAŽETAK:

Uprkos kontraverznim i često nepotpunim literarnim podacima uređaj za aspiracionu trombektomiju se sve više koristi u interventnoj kardiologiji tokom svakodnevne kliničke prakse. Predstavljamo interesantan prikaz slučaja akutnog koronarnog sindroma (AKS) kod pacijenta koji se lečio od hronične inflamatorne bolesti creva. Tokom dijagnostičke kolonoskopije u regionalnoj bolnici dolazi do razvoja infarkta miokarda sa ST elevacijom (STEMI), atrijalne fibrilacije i srčanog popuštanja. Interventni tretman kod ovakvih kompleksnih pacijenata uvek predstavlja izazov.

Ključne reči: *akutni infarkt miokarda, aspiraciona trombektomija*

INTRODUCTION

We report a case of a female patient who presented with acute coronary syndrome (ACS) during rectoscopy in a regional hospital due to suspicious inflammatory bowel disease. Due to exacerbation of inflammatory bowel disease, she had not received fibrinolytic therapy. She was transferred to our institution for coronary angiography, which revealed a transparent filling defect at the bifurcation of the first obtuse marginal branch (OM1), which is highly suggestive of an intra-arterial thrombus. Therefore, we decided to treat the OM1 branch with a QuickCat thrombectomy device. The aspirated material was a Y-shaped thrombus, which corresponded well to the angiographic image of the bifurcated thrombus.

Case report

During a rectoscopy at a regional hospital, aA 66-year-old woman presented with acute coronary syndrome (ACS) and acute heart failure (Killip class II) that manifested as STEMI at a,due to suspicious inflammatory bowel disease. She had history of a previous posterior myocardial infarction (MI) in 2004. Her ECG had showed posterolateral MI with atrial fibrillation. Due to exacerbation of the inflammatory bowel disease, she had not received fibrinolytic therapy. In the following days, she presented with prolonged anginal episodes, electrical instability, and heart failure. She had been defibrillated due to ventricular fibrillation (VF). Then, she was transferred to our institution for coronary angiography, which revealed borderline

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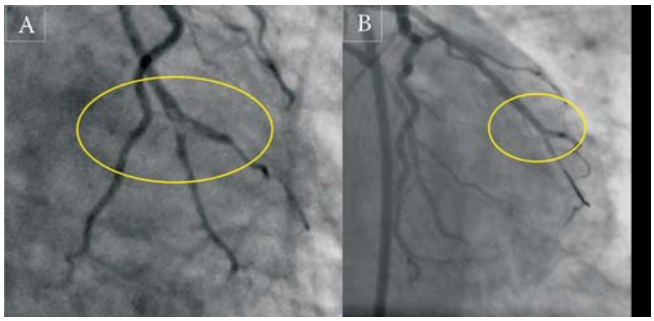

Fig. 1. Filling defect on the angiogram of the obtuse marginal artery (A) and its disappearance after thrombectomy (B).

Fig. 2. Thrombus specimen

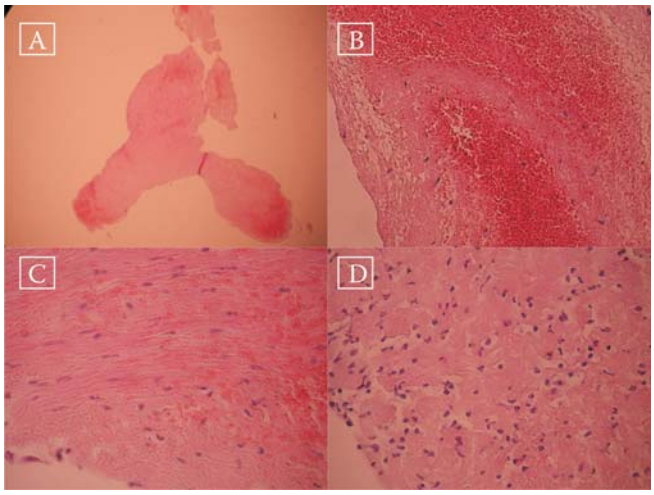

Fig. 3. Pathohistological characteristics of the aspirated thrombus

A - Macroscopic appearance of the Y-shaped specimen (40x magnification)
B - A major part of the specimen was composed of an eosinophilic mass, with the border zone dominated by fibrin, erythrocytes, platelets and rare granulocytes (200x magnification)
C - Some parts had spindle cells (400x magnification)
D - The central parts were homogenised, revealing zones containing cytologically changed granulocytes (400x magnification)

disease in the right coronary artery (RCA), a relatively disease-free left anterior descending artery (LAD), 95% in mid-Cx, which suggested an old lesion, and a transparent filling defect at the bifurcation of first obtuse marginal branch (OM1), which is highly suggestive of an intra-arterial thrombus (Fig. 1A). Therefore, we decided to treat the OM1 branch first with a QuickCat thrombectomy device. The aspirated material was a Y-shaped thrombus that corresponded well to the angiographic image of the bifurcated thrombus (Fig. 2). Control angiography revealed no underlying stenotic lesions (Fig. 1B). The ST elevations in the lateral precordial leads and chest pain immediately resolved, so we decided to finish the procedure. Before discharge, an echo examination only revealed the hypokinetic posterior and lateral wall of the left ventricle, with moderate a global enlargement and an ejection fraction (EF) of approximately 50%.

After 24-hour fixation in a 4% solution of formaldehyde, the aspirated thrombus was embedded in a paraffin cast. Five-micron slices (16 levels of cuts) were dyed by standard haematoxylin and eosin stain. Microscopic analysis revealed a mixed thrombus structure. A major part of the tissue was composed of an eosinophilic mass, with the border zone dominated by fibrin, erythrocytes, platelets and rare granulocytes. The central parts were homogenised, with zones in which cytologically changed granulocytes were appreciated , and some parts had spindle cells as well (Fig. 3).

The patient left the hospital in good overall condition, on double antiplatelet therapy, but after 4 weeks, she was again admitted to a local hospital due to acute heart failure. Her cardiac ultrasound examination showed normal inferior wall kinetics but posterior wall hypokinesis, and she was transferred to our centre again for a circumflex artery PCI procedure, which proceeded uneventfully. New episodes of atrial fibrillation were recognised, and anticoagulant therapy was added.

DISCUSSION

It has been reported that occlusive thrombi are composed of platelets, fibrin, erythrocytes and leucocytes [1], but they are predominantly composed of platelets in STEMI patients [2]. Murakami et al. classified thrombi according to thrombus age into three groups: fresh, lytic and organised. Fresh thrombi are one day old and are mainly constituted of fibrin, erythrocytes, platelets and intact granulocytes [3]. The second group, lytic thrombi, are between one and five days old and thrombi are characterised by zones of cytologically changed granulocytes [3]. The third group, organised thrombi, is characterised by the presence of spindle cells and; are older than five days. and They may,, comprise connective tissue and capillaries, but this is not required [3]. Thrombi with heterogeneous appearances of fresh, lytic and organised thrombi are always classified as older than five days. Therefore, the extracted

mass in our case belongs to the last group. It is assumed that the heterogeneous nature of thrombi results from episodes of thrombus growth before the onset of occlusive thrombosis and clinical symptoms. Pathohistological analysis of thrombectomy samples from patients with acute myocardial infarction has shown that, in primary PCI, older thrombi were found in as many as 50% of patients [4]. They obstruct the infarct-related artery but embolise downstream micro-vessels, by mechanical fragmentation, which is either spontaneous or introduce via interventional procedures with eithera balloon or stent.

There are data [5] that confirm a high risk of thrombosis and markedly abnormal platelet function in patients with inflammatory bowel disease,. Both abnormalities are unrelated to the underlying bowel disease activity, and they imply that platelet hyperaggregation may be important in the development of thrombosis in such patients. The American Society for Gastrointestinal Endoscopy has published guidelines on the management of anticoagulants during endoscopy [6]. Their risk stratification model for endoscopic procedures was adopted to stratify the risk of haemorrhage as well asand certain clinical situations that could result in a high risk of thromboembolic complications. , Based on the study by Keeley et al., (2003) published in *Lancet* , we can assume that an interventional strategy is preferred to thrombolytic therapy in patients presenting with STEMI [7]. One can postulate about the importance of epicardial artery patency, the level of myocardial perfusion, reperfusion injuries and their predictive values on definite clinical outcomes [8]. There is, of course, the additional role of the microvascular response to thromboembolisation, including neurohumoural and vasospastic responses. We have to deal with more or less frequent, angiographically visible thrombotic material in the setting of STEMI. Disposing of this material is ideal. It is logical to expect that patients with the largest thrombus burden benefit most from thrombus extraction.

Thrombus-containing lesions result in seven-fold increases in hard endpoints, namely periprocedural myocardial infarction, emergency coronary artery bypass grafting (CABG) and death [9]. There is evidence that not all thrombi are visible on angiography —only one-third, according to angioscopic data [10]. Essentially, two general principles have motivated investigators and researchers in the medical community and in industry . The first is to evacuate thrombi out of the artery, and the second is to protect against further embolisation during interventional procedures. It seems that different extraction catheters and systems outnumber so-called protective devices, filters, occlusive balloons, etc. Despite all of those efforts, there is still no firm evidence from randomised, multicentred, clinical trials that favour the use of protective devices in STEMI over simple interventional approaches [11].

All of these methods showed better results in selected populations of AMI patients, particularly according to ST segment resolution rate or angiographic parameters, such as MBG (myocardial blush grade) or TIMI flow, compared to the conventional interventional approach. However, the 30-day mortality rate did not differ significantly.

Nevertheless, it seems logical that the device should be as user-friendly as possible, as well as simple and preferably cost-effective. At the same time, it must be safe, particularly smooth in delivery and not dissective or even perforative in nature. At the same time, the efficacy of such a device is usually measured using angiographic flow parameters, which act as surrogate markers for clinical efficacy, and clinical efficacy is truly the most important value that is measurable only through longer follow-up periods in large randomised trials.

Overall, there have been several meta-analyses published about the role of thrombectomy devices in primary or rescue PCI, and they are all concordant in their conclusions that there were no clinical benefits in terms of lower 30-day mortality rates. It ishould be stressed, however, that most complex and high-risk patients were excluded from these trials. Therefore, overall mortality would have been low anyway. New results from the TAPAS trial, which was the first major trial in 15 years, showed that mortality was altered by an adjunctive intervention [12].

Although mechanical removal of larger thrombus burdens before PCI reduces a source of potential embolisation, it does not prevent further aggregation during or shortly after the interventional procedure [12]. The vacuumed material is supposed to be macerated and dissolved during suction and collection in the drainage syringe; did not however, in our case, in which the thrombus sustained its macroscopic characteristics, despite a being suctioned through a narrow pathway. There are also endogenic fibrinolytics and anti-thrombotic agents that all contribute to the is lack of clear evidence of thrombi in collected blood samples from infarct-related arteries; yet, after catheter aspiration, we sometimes cannot appreciate thrombi in the vessel anymore. Soft material, i.e., red thrombi, is unlikely to be suitable for examination. However, white thrombi are somehow more difficult to aspirate through the relatively small lumen of aspiration catheters. The success of catheter aspiration is determined by the deliverability of the catheter, which depends on lesion localisation, vessel compliance, calcification and tortuosity.

This case shows the optimal performance of anthe available, easy-to-use, QuickCat device for a very quick and simple embolectomy procedure in the setting of acute myocardial infarction.

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