SIMTI recommendations on blood components for non-transfusional use

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Introduction

Recommendations are a way of distilling a large amount of scientific information into a format that can be easily used by a doctor and that is applicable to an individual patient. According to an authoritative definition, recommendations are "indications on clinical behaviour, produced through a process of systematic review of the literature and experts' opinions, with the aim of helping doctors and patients to decide the most appropriate care in specific clinical situations"[1].

Recommendations are, therefore, prepared with the purpose of ensuring the highest level of appropriateness of interventions and minimising that part of variability in clinical decisions related to lack of knowledge and subjectivity in the definition of care strategies. Legislative decree 229/99 and the National Health Plan for 1998-2000 and subsequent versions proposed the adoption of recommendations as a way to improve the efficient and effective use of available resources and the appropriateness of prescriptions[2,3].

The expected results concern:

- the user, who can be better informed and more aware of the scientific reasoning supporting the treatments received;
- the hospitals, which can define and optimise their care processes and, therefore, plan their own expenditure;
- various institutions at different levels (State, Region, Healthcare Authority), which can reduce inequalities in the allocation of services and facilitate the monitoring and evaluation of the quality of the services supplied;
- professionals, for whom the recommendations are a means of continuing medical education, but which can also improve the relationship between healthcare staff and citizens-patients and protect against medico-legal risks.

Methodology of the Working Group and grades of recommendation

The process of developing these Recommendations, as for those already published by SIMTI and in accordance with the indications contained in the methodology manual of the national guidelines system (SNLG)[4], was based on systematic reviews of the literature. At a later stage the recommendations will be discussed in a multidisciplinary context and with the relevant institutional bodies. An evaluation is included of the quality of the proof and the strength with which each of the single recommendations is made[4].

The methodology used to prepare the grades of recommendation was based on that adopted at the Consensus Conference of the American College of Chest Physicians in 2004[5], thereby privileging similarity and uniformity with the other Recommendations published in recent years by SIMTI, rather than using the more recent methodological criteria which, although similar, give less weight to the role of consistent, unequivocal observational studies.

The recommendations are classified by *grades,* expressed in Arabic numbers (*1, 2*), according to their strength, and in *letters* (*A, B, C*), according to the evidence and type of study.

In detail (Table I - Appendix):

Grade 1: the authors are certain that the benefits are greater or less than the costs in terms of risk and financial expenditure. This is, therefore, a *strong recommendation*.

Grade 2: the authors are less certain concerning the points above and, therefore, make a weaker recommendation.

As far as concerns the classification by letters:

Grade A: the recommendation derives from the evidence of numerous, consistent randomised studies.

Grade C+: the recommendation derives from the analysis of observational clinical studies, but with very consistent results, or from results that can be unequivocally extrapolated from randomised studies.

Grade B: the clinical studies providing the evidence were randomised, but had important limitations (discordant results, methodological flaws).

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Grade C: the recommendation derives from an analysis of observational studies, with less consistent results, or from results extrapolated with a lower degree of certainty from randomised studies; recommendations based on clinical experience/opinion of experts are also classified as grade C.

The verb "*recommend*" is used for the higher grades (1A, 1C+, 1B, 1C), while the verb "*suggest*" is used for the lower grades (2A, 2C+, 2B and 2C).

In general, any recommendation other than Grade 1A implies that the authors recognise that there are alternative interpretations of the available evidence and other clinical policies can be reasonably considered appropriate. Furthermore, even the Grade 1A recommendations may not be indiscriminately applicable in every circumstance and in every patient.

The conventional classification of evidence is based on mathematical and statistical criteria, assigning the "strength" of the evidence, in decreasing order, to: meta-analyses, randomised controlled trials, retrospective studies, prospective follow-up studies, cross-sectional population studies, reviews and anecdotal reports. This is correct as far as regards strictly clinical studies, especially if they are therapeutic investigations focused on the evaluation of objective outcomes.

Nevertheless, the recommendations are weak in some fields, whereas in others, the availability of clinical trials carried out with rigorous methodology in large populations of subjects has enabled specific, more certain recommendations to be made.

This document will be periodically revised in the light of new scientific information that becomes available.

Each member of the Working Group signed a declaration, in conformity with that adopted by the SNLG, that they did not have any conflicts of interest regarding these recommendations.

Blood components for non-transfusional use

The use of blood components for non-transfusional purposes started in 1998 with the first report by Marx *et al.*[6] on the application of platelet concentrates in dentistry. In the wake of the first clinical successes, interest in the concept broadened to various fields of medicine and surgery; numerous production methods and a variety of indications for use were proposed and the practice spread in various specialities.

Among the various blood components for non-transfusional use, *platelet concentrate for non-transfusional use* has gained a central role. This concentrate is a source of growth factors and is used, both in liquid and activated forms, to promote the regeneration of damaged tissues.

Data published in the literature already several years ago showed that platelet gel was effective in the treatment of various disorders, such as skin ulcers, with relief of pain, reduction in inflammation, increase of angiogenesis and stimulation of granulation tissue[7-9].

The effect of platelet concentrates of stimulating the regeneration of bone and soft tissues led to these blood components being used in other clinical settings, in particular in maxillo-facial surgery, in dentistry (dental implants, sinus augmentation, cleft palate), orthopaedics and traumatology (soft tissues lesions, non-union, loss of bone substance following trauma or excision of cysts), in ophthalmology (lesions to the corneal epithelium), heart surgery (sternal wound dehiscence) and in other specialities following numerous reports which, although based on methodologically limited studies and small populations, suggest that the product is effective, easy to use and does not cause adverse events or reactions[10].

The mechanisms of action of platelet concentrate for non-transfusional use in cell regeneration and in the stimulation of tissue repair have not yet been fully identified. It is known that the mechanisms involved are more complex than thought until recently; platelets contain more than 300 proteins and the application of new diagnostic techniques in molecular biology could aid better identification of the mechanisms of transmission of biochemical signals involved in tissue regeneration.

Organisational framework and regulatory aspects

General principles

Allogeneic or autologous blood components for non-transfusional purposes can be produced: (i) for surface use on skin or mucosae (topical use); (ii) for infiltration into tissues; (iii) as material to apply locally to surgical wounds, alone or with non-cellular biological material (e.g., banked bone tissue) or with medical devices.

The criteria and principles for requesting, evaluating appropriateness, assigning, delivering and safety monitoring are the same for these products as for those for blood components administered by transfusion. Identification and traceability must be ensured using the computerised management system in function in the Transfusion Service.

Apart from specific exceptions, defined in this document, related to the need for repeated applications and/or simplicity of the applications and/or production methods:

- the request for blood components for non-transfusional use must be made by a doctor or a dentist (only for the clinical activities of his competence);
- blood components for non-transfusional use must be produced within Transfusion Services or their organisational structures; only autologous blood

components can also be produced outside of Transfusion Services, in accordance with the criteria and constraints explained further on;
- the blood components for non-transfusional use shall be applied by a doctor, or by a nurse under the control and responsibility of the doctor;
- blood components used in dentistry shall only be applied by a dentist for the clinical activities of his or her competence;
- the allogeneic or autologous blood components shall be stored in Transfusion Services or in their organisational structures and the storage shall comply with the same criteria as those applied to blood components used for transfusion.

Products containing autologous or allogeneic stem cells intended for administration by a non-transfusional route are not covered by this document since they may be, or are to all effects, "advanced therapy medicinal products". In fact, pursuant to Regulation (EC) N. 1394/2007 of the European Parliament and of the Council of 13 November 2007[11], cells or tissues are considered tissue engineered products (and, therefore, advanced therapy medicinal products) if they have been subject to substantial manipulation or if they are not intended to be used for the same essential function or functions in the recipient as in the donor. For the purpose of defining advanced therapy medicinal products, the Regulation therefore emphasises the concept of non-homologous use (intended in this setting as the use of cells in the recipient for functions differing from those that the cells had in the donor, whether autologous or allogeneic), but does not distinguish between allogeneic use (donor and recipient are different people) and autologous use (donor and recipient are the same person)[11-13].

SIMTI considers, also for the sake of prudence, that this matter should be subject to the specific legislation on advanced therapy medicinal products (and, therefore, to the specific evaluations of and authorisations by the Italian National Institute of Health [ISS] and the Italian Drug Agency [AIFA]) and not to the legislation strictly applicable to transfusions.

For the production and application of blood components for non-transfusional use based on methods and principles other than those set out in this document, a specific clinical research project, drawn up jointly by the Transfusion Service and the health facility user, must be prepared for submission to the relevant Ethical Committee for the committee's authorisation.

Products that can be used by a non-transfusional route

Platelet concentrate (allogeneic or autologous)

Platelet concentrate can be prepared from the donation bag of whole blood, from an apheresis donation or from a blood sample collected into a specific device. It has a defined platelet content and variable volume, depending on its intended use. Thrombin, as an accessory of the platelet concentrate, can be produced at the same time as the production of the platelet concentrate. The platelet concentrate can be used fresh or after freezing.

Platelet gel (allogeneic or autologous)

This is obtained starting from platelet concentrate, following activation of the coagulation cascade. It is usually produced at the site of application; it can be generated during the production stage and delivered already ready for use, fresh or after freezing.

Eye drops from autologous serum

These are produced starting from a sample of blood in which coagulation is activated and the serum component separated.

Eye drops from platelet concentrate

These are produced from autologous platelet concentrate and then subjected to lysis.

Methods of sample collection and production

Depending on the type of use, the volumes required and the possibility/necessity of freezing the product, blood components for non-transfusional use may need to be collected using devices other than the normal systems for the collection of blood and blood components (e.g., small volume bags, ad hoc devices, tubes in which to produce the blood component or activating plasma factors then destined for use on the patient).

In all cases the collection, production and application of blood components for non-transfusional use must be carried out using CE-marked devices for the specific purpose, in accordance with Directive 93/42/EC (class IIa or higher).

Phases of production in the open must be avoided or minimised; if processing must take place in the open, measures must be taken to ensure the sterility of the product (sterile connections, processing under a laminar flow hood).

Allogeneic blood components for non-transfusional use

Allogeneic blood components for non-transfusional use:
- must be produced only within Transfusion Services or their organisational structures;
- are obtained exclusively from normal donations of blood or blood components, subjected to the same procedures of biological validation;
- may undergo specific processing, division into aliquots, and treatments;
- must be labelled with the world donation code of the donation, type of blood component and, if divided into aliquots, the identification of the aliquot.

Autologous blood components for non-transfusional use

Autologous blood components for non-transfusional use:

- should be produced within Transfusion Services or their organisational structures; the production may take place outside Transfusion Services to the extent and in the manner specified further on;
- should be obtained from patients who are not at risk of bacteraemia;
- may be produced using different methods of collection, that is, with different types of medical devices and in different volumes; the volume of anticoagulant must be commensurate with the amount of blood taken;
- for withdrawal of volumes greater than 200 mL the patient must meet the criteria for eligibility for pre-deposit autologous donations;
- in the case of withdrawal of volumes greater than 300 mL, an evaluation must be made of whether it would be appropriate to re-infuse the red cell component;
- can be subjected to specific processing, division into aliquots, and treatments;
- are subject to the same criteria and procedures for identification, registration, segregation and traceability as units of autologous pre-deposited blood.

As far as concerns the performance of tests of biological validity of autologous blood components for non-transfusional use:

- the tests of biological validation should be performed at the start of a therapeutic cycle and are valid for a maximum of 30 days;
- the tests of biological validation may be omitted if the collection, production and application of the autologous blood component takes place within a single session without any preservation of the product.

Autologous eye drops

In relation to the need for frequent applications and simplicity of administration, autologous eye drops (eye drops from autologous serum or platelet concentrate) may be stored at home provided that:

- they are produced in Transfusion Services or their organisational structures, using specific medical devices and according to production procedures that guarantee the sterility of the products;
- they are packed as single-dose, disposable products;
- every dose is suitably identified;
- the duration of storage at home is less than 30 days;
- the patient is appropriately informed and trained with regards to the storage and self-administration and that there is documentation to this effect. The specialist oculist who requested the autologous eye drops is responsible for training the patient with regards to their storage and administration.

The Transfusion Service that produced the eye drops delivers them to the specialist oculist who requested them (or to a person specifically delegated by the oculist).

Packaging and transport

Prior to delivery, blood components for non-transfusional use must be packaged in a container suitable for transport that guarantees the product's integrity and insulation. Depending on the size, an ad hoc container can be used. An appropriate identification label must be applied to the blood components.

The delivery must be accompanied by appropriate, specific forms similar or identical to those for the delivery of standard blood components; thus, the form must identify the product and contain the recipient's general information, the method of preparation and storage of the product, the times of use and/or shelf-life.

There must be a form to return to the Transfusion Service in which the doctor who used the product records its application and any adverse events or reactions experienced by the patient.

With regards to transport, the hygiene and size of the containers and the time for arrival at destination must be guaranteed.

Collection/production of autologous blood components for non-transfusional use outside of Transfusion Services

The phase of collecting/producing autologous blood components for non-transfusional use can be performed outside Transfusion Services or their organisational subunits provided that:

- the relevant regional bodies have issued general or specific authorisation for this;
- a specific agreement has been stipulated pursuant to Ministerial Decree of 1st September, 1995 and any specific regional regulations, between the healthcare structure that intends to make use of this possibility and the hospital in which the Transfusion Structure is located;
- the procedures for producing and using the products are based on scientifically recognised, standardised therapeutic protocols;
- the volume collected in a single procedure does not exceed 60 mL and the overall volume taken in a cycle of procedures does not exceed 300 mL in 90 days;
- the collection, production and application are all planned for a single session with no storage of the product;
- the methods of applying the product are defined.

The agreement must define:
- where the activities will be carried out and the characteristics of the buildings in which these activities will be performed;
- what products will be produced, with what methods and using which medical devices and equipment (contracts and maintenance programmes);
- the disorders that will be treated and the aims of the use of the products;
- the methods of identifying the procedures and the products;
- the methods of recording the procedures and products in their place of production;
- the methods of sending the reference Transfusion Service a periodic summary report (at least twice yearly) of the patients who have undergone a procedure, of the products obtained and of the use of the products;
- a model of the informed consent form for the procedure, examinations and the management of the results of the examinations;
- a contact doctor (or dentist in the case that the product will be used in a dental setting) responsible for the activities in the healthcare facility that has stipulated the agreement;
- the professional figures and names of the people at the healthcare facility who will, after appropriate education and training, carry out the different stages of the process (evaluation of the patient, collection of sample, production, application, registration);
- the methods of educating and training the person in charge at the healthcare facility and the various healthcare workers involved in the different phases, bearing in mind that the reference Transfusion Service has the duty to and responsibility of certifying the training and its efficacy (any training by companies supplying medical devices should be considered an integration of the training by the transfusion structure);
- that the blood components must be applied in the same structure in which they are produced and that they are used immediately after production (it is forbidden to store the blood components in the healthcare structures in which they are used);
- the arrangements for monitoring reactions, serious adverse events and accidents, as well as the information flow for reporting them and notifying the relevant regional authorities and SISTRA (the Italian national Transfusion Services Information System);
- the frequency (at least annually) and the arrangements for the audits by the Transfusion Service and for the controls of the production process and final product (sterility, platelet content);
- administrative relations between the two structures with regards to maintenance and validation of the production equipment, audits, and valorisation of the production processes;
- that every change to the production process as well as changes in use must be reviewed together with the Transfusion Service, and must be approved by it before being introduced.

Production of blood components for non-transfusional use

Methods of producing platelet concentrate for non-transfusional use

The products currently available can be divided into:
- products prepared by the Transfusion Structure starting from autologous or allogeneic donations;
- autologous products obtained using *specific medical devices* (collection sets, tubes, activators, centrifuge, etc.) for the specific preparation of the given product.

Platelet concentrate for non-transfusional use

This is prepared from anticoagulated blood (ACD, CPD, sodium citrate) in different volumes depending on the planned use and contains platelets re-suspended in plasma. It can be used fresh or after having been frozen and contains $1\times10^6\pm20\%$ platelets/μL.

The amount of leucocytes present depends on the method used for its preparation.

The sterility of the product is guaranteed by suitable validation of the production process.

Allogeneic platelet concentrates for non-transfusional use

Allogeneic platelet concentrates for non-transfusional use can be prepared from donations of whole blood, or by apheresis according to standard procedures for the preparation of blood components and must undergo the same procedures of biological validation. The platelets must be suspended in plasma in order to guarantee the full range of plasma proteins.

The product can be divided into aliquots of various volumes which can then undergo specific processing and treatments and, if necessary, be stored.

Autologous platelet concentrates for non-transfusional use

These platelet concentrates can be prepared from whole blood, from apheresis, or using other methods of collection, such as tubes or various types of medical devices, and in different volumes; the volume of anticoagulant must be proportional to the amount of blood collected.

Preparation with specific devices. The blood collected is processed according to the specifications of the system used in order to obtain the optimal content of platelets ($1\times10^6/\mu L\pm20\%$) that remain suspended in the plasma; if some of the phases of the preparation are performed in an open system, appropriate measures must be taken to ensure the sterility of the product.

Products obtained from processing platelet concentrates for non-transfusional use

Platelet gel

Platelet concentrate for non-transfusional use, obtained by one of the procedures described above, is activated to obtain a semi-solid product. The activation is induced by:

- calcium chloride or gluconate;
- calcium chloride or gluconate and human thrombin;
- calcium chloride or gluconate and batroxobin®;
- calcium chloride or gluconate and centrifugation.

It can be used fresh or after having been frozen.

Preparation of autologous or allogeneic thrombin

This is obtained by re-calcification of the plasma. After addition of calcium (chloride or gluconate) in a ratio of 1:5, the plasma is left to clot for about 30 minutes at room temperature of at 37 °C.

Once the clot has formed, the plasma enriched in thrombin is ready to use (after squeezing the clot/high-velocity centrifugation); it can be divided into aliquots.

Use of the thrombin

The thrombin, correctly and univocally identified, can be delivered to the user for the activation of the platelet concentrate at the moment of its application.

Platelet concentrate - eye drops

The platelet concentrate for non-transfusional use obtained by one of the procedures described above is subjected to cyclical freezing/thawing and subsequent high velocity centrifugation; the supernatant is diluted with a volume of physiological saline or balanced saline solution (BSS) equivalent to at least 30% of the volume of the supernatant. The doses must be prepared using procedures that ensure the sterility of the product. It can be used fresh or frozen.

Eye drops from autologous serum

These are prepared from clotted whole blood. The volume of blood collected depends on the amount needed. The serum is diluted with a volume of physiological saline or balanced saline solution (BSS) equivalent to at least 30% of its volume. The doses must be prepared using procedures that ensure the sterility of the product. It can be used fresh or frozen.

Storage of blood components for non-transfusional use

Blood components for non-transfusional use must be stored according to the procedures already standardised for the storage of blood components for transfusions:

- storage at room temperature for a maximum of 6 hours;
- storage at a temperature below −25 °C for a maximum of 24 months;
- storage at a temperature between −18 °C and −25 °C for a maximum of 3 months.

Therapeutic indications for blood components for non-transfusional use

When defining the indications for the use of blood components for non-transfusional use, it should be highlighted that, since 1998, multiple therapeutic initiatives have developed which, although having a clinical rationale, do not fulfil the criteria of "evidence-based medicine". It is, therefore, important to consider the strictly medico-surgical fields of application that are based on consistent, convincing literature, in the awareness that, so far, there are few meta-analyses, study reviews and randomised clinical trials of sufficient size to provide significant clinic-therapeutic guidelines.

The review of the literature was performed by including some restrictions aiming at filtering out studies that were of little significance because of their type, methodology or population size. In detail, the following research criteria were adopted when searching the main data banks available:

- controlled clinical trials (CCT) and randomised clinical trials (RCT);
- meta-analyses;
- Cochrane Library reviews;
- studies published in the last 5 years;
- more than 20 patients in the study.

Wounds

Diabetic ulcer

The studies considered with regards to this indication were a substantial systematic review[14] (which analysed 18 studies of which 16 were evaluated: seven RCT, three cross-sectional studies, one retrospective, multicentre study, one multicentre, case-control study and four studies without a control group for a total of over 30,000 patients; furthermore, four trials considered methodologically similar were evaluated in a meta-analysis) and two randomised studies[15,16].

Although some questions have been raised considering the comparability of the studies and the risk of bias between the series analysed[17], overall the use of platelet concentrate in diabetic ulcers was found to be the treatment of choice.

Grade of recommendation: 1B

Chronic ulcers and difficult wounds

The studies that could be evaluated were an observational study with a large population and positive clinical results in a high number of patients[18], as well as a meta-analysis of 24 clinical studies, conducted in the last 10 years, in which it was concluded that PRP accelerated the process of healing of chronic ulcers and difficult wounds[19].

Grade of recommendation: 1C

Musculoskeletal system

The use of infiltrated blood components for non-transfusional purposes in the context of orthopaedics has increased recently, as witnessed by the substantial quantity of publications on the subject in the last few years. In this field 11 RCT/CCT were taken into consideration[20-30] of which four came to positive conclusions and seven did not show significant differences compared to the control groups. It should be noted that two of the four studies that reached positive conclusions concerned the treatment of epicondylitis in a total of 130 patients with a follow-up of 2 years[21,22]. The conclusions of a recent meta-analysis are particularly interesting[31]: an analysis of 23 RCT and ten prospective clinical studies did not reveal clear evidence in favour of the clinical use of platelet concentrates in bone or soft tissue lesions in the field of orthopaedics. It is worth noting the comment concerning the lack of standardisation of the production methods, dose regimens and criteria for use[32]. Furthermore, it should be pointed out that, for some pathologies, only one study meeting the previously described criteria was available for evaluation.

Infiltration treatment of epicondylitis

Grade of recommendation: 1B

Treatment of lesions of the rotator cuff

Grade of recommendation: 2B

Treatment of lesions of the anterior cruciate ligament

Grade of recommendation: 2B

Treatment of lesions of Achilles' tendon

Grade of recommendation: 2C

Other disorders of bone, muscles or ligaments

Grade of recommendation: 2C

Dentistry and maxillo-facial surgery

In this context the literature seems to be particularly controversial and the RCT/CCT evaluated, carried out on limited numbers of patients, included studies supporting the use of the blood components for non-transfusional use and other studies not indicating significant differences from the control groups[33-38].

Two recent reports seem to be particularly interesting:

- a Cochcrane database systematic review in 2010[39], which concluded that there was a lack of evidence of improvement of clinical outcomes in augmentation procedures of the maxillary sinus treated with platelet concentrate for non-transfusional use;
- the meta-analysis by Bae *et al.* in 2011[40], which showed the efficacy of platelet concentrate for non-transfusional use in bone grafting for maxillary sinus reconstruction with a view to implants.

Treatment for maxillary sinus augmentation

Grade of recommendation: 2B

Other dental disorders

Grade of recommendation: 2B

Ophthalmology

Platelet concentrate - eye drops

The studies in the literature seem to be very limited regarding the types of conditions treated and the sizes of the populations studied. One RCT (35 patients)[41] and one CCT (38 patients)[42] could be evaluated: both involved corneal disorders (alkali burns and ulcers) and the positive results that emerged could not be considered conclusive.

Chemical burns of the ocular surface

Grade of recommendation: 2B

Corneal ulcers

Grade of recommendation: 2B

Eye drops from autologous serum

No RCT or CCT meeting the selection criteria was identified; the only published studies were uncontrolled studies or case reports on "dry eye syndrome".

Grade of recommendation: 2C

At present there are no clinical studies in other fields or for other indications that meet the criteria adopted for these Recommendations.

Finally, as far as concerns the methods of use, dose regimens, frequency and duration of treatment, reference should be made to clinical protocols agreed by specialists in the specific sector.

The Authors declare no conflict of interest.

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Appendix

Table I - Grades of recommendation[1].

Grade of recommendation	Clarity of evidence on the risk/benefit ratio	Methodological strength	Implications
1A	Clear	Randomised controlled trials without important limitations.	Strong recommendation; applies to most patients in most circumstances without reservations.
1C+	Clear	No randomised clinical trials but strong results can be extrapolated from randomised clinical trials, or overwhelming evidence from observational studies.	Strong recommendation; applies to most patients in most circumstances.
1B	Clear	Randomised studies with important limitations (inconsistent results, methodological flaws).	Strong recommendation; probably applies to most patients.
1C	Clear	Observational studies	Intermediate strength recommendation; may change when stronger evidence is available.
2A	Unclear	Randomised controlled trials without important limitations	Intermediate strength recommendation; best action may differ depending on circumstances or patients' or societal values.
2C+	Unclear	No randomised clinical trials but strong results can be extrapolated from randomised clinical trials, or overwhelming evidence from observational studies.	Weak recommendation; best action may differ depending on circumstances or patients' or societal values.
2B	Unclear	Randomised studies with important limitations (inconsistent results, methodological flaws).	Weak recommendation; alternative approaches likely to be better for some patients under some circumstances.
2C	Unclear	Observational studies, the opinions of authoritative experts or committees of experts or the Working Group responsible for these recommendations.	Very weak recommendation; other choices may be equally reasonable.

Table II - Elements for evaluating the references.

Ref. #	Authors	Year	Title	Journal	Type of study	Subject	Follow up	N. of pts	Results (***Bold Italic: study with a positive result***; *Italic: study with a negative result*)
14	Villela DL, Santos VL	2010	Evidence on the use of platelet-rich plasma for diabetic ulcer: a systematic review.	Growth Factors 2010; **28**: 111-6.	Systematic review	Diabetic ulcers	NA	30,429	***Evidence of a favourable outcome from using PRP in diabetic ulcers***
15	Saad Setta H, Elshahat A, Elsherbiny K, et al.	2011	Platelet-rich plasma versus platelet-poor plasma in the management of chronic diabetic foot ulcers: a comparative study.	Int Wound J 2011; **8**: 307-12	RCT	Diabetic foot ulcers	20 weeks	24	***Use of PRP speeds healing of chronic diabetic foot ulcers.***
16	Driver VR, Hanft J, Fylling CP, et al.	2006	A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers.	Ostomy Wound Manage 2006; **52**(6): 68-70,72,74.	RCT	Diabetic foot ulcers	12 weeks	129	***Autologous PRP promotes healing of diabetic foot ulcers***
17	Cochrane Library Centre for Reviews and Dissemination	2012	Evidence on the use of platelet rich plasma for diabetic ulcer: a systematic review (Structured abstract).	Database of Abstracts of Reviews of Effects 2012 Issue 1	Systematic review and meta-analysis	Diabetic ulcers	NA	NA	***PRP is the therapy of choice for topical treatment of chronic diabetic ulcers.***
18	De Leon JM, Driver VR, Fylling CP, et al.	2011	The clinical relevance of treating chronic wounds with an enhanced near-physiological concentration of platelet-rich plasma gel.	Adv Skin Wound Care 2011; **24**(8): 357-68.	CCT	Chronic ulcers	22 weeks	200	***Positive clinical response to the use of PRP in 96.5% of ulcers treated (275 of 285).***
19	Carter MJ, Fylling CP, Parnell LK.	2011	Use of platelet rich plasma on wound healing: a systematic review and meta-analysis.	Eplasty 2011; **11**: e38	Systematic review and meta-analysis	Chronic ulcers	NA	NA	***Partial and complete healing of ulcers treated with PRP improved compared to the control group***
20	Randelli P, Arrigoni P, Ragone V, et al.	2011	Platelet rich plasma in arthroscopic rotator cuff repair: a prospective RCT study, 2-year follow-up.	J Shoulder Elbow Surg 2011; **20**(4): 518-28	RCT	Rotator cuff	2 years	53	***The use of PRP reduces pain in the early months of the post-operative period.***
21	Gosens T, Peerbooms JC, van Laar W, den Oudsten BL.	2011	Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis: a double-blind randomized controlled trial with 2-year follow-up.	Am J Sports Med 2011; **39**(6): 1200-8.	RCT	Epicondylitis	2 years	100	***PRP in patients with chronic lateral epidcondylitis significantly reduces pain and improves function.***
22	Hechtman KS, Uribe JW, Bottovan Demden A, Kiebzak GM.	2011	Platelet-rich plasma injection reduces pain in patients with recalcitrant epicondylitis.	Orthopedics 2011; **34**(2): 92.	CCT	Epicondylitis	1 year	30	***Single doses of PRP improve function score and pain, avoiding the need for surgery***
23	Radice F, Yanez R, Gutiérrez V, et al.	2010	Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors.	Arthroscopy 2010; **26**(1): 50-7.	CCT	Anterior cruciate ligament (ACL) lesions	1 year	50	***Used during the reconstruction of ACL lesions, PRP shortens the healing time by 48% compared with the controls.***

Legend RCT: randomised clinical trial; CCT: controlled clinical trial; NA:not applicable; ND: non available.

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Table II – Elements for evaluating the references.*(continued from previous page)*

Ref. #	Authors	Year	Title	Journal	Type of study	Subject	Follow up	N. of pts	Results (***Bold Italic: study with a positive result***; *Italic: study with a negative result*)
24	Silva A, Sampaio R.	2009	Anatomic ACL reconstruction: does the platelet-rich plasma accelerate tendon healing?	Knee Surg Sports Traumatol Arthrosc 2009; **17**(6): 676-82.	CCT	Anterior cruciate ligament lesions	3 months	40	*No difference in magnetic nuclear resonance images between controls and subjects treated with PRP.*
25	Nin JR, Gasque GM, Azcárate AV, et al.	2009	Has platelet-rich plasma any role in anterior cruciate ligament allograft healing?	Arthroscopy 2009; **25**(11): 1206-13.	RCT	Anterior cruciate ligament lesions	2 years	100	*The use of PDGF in patients treated with bone-patellar tendon grafts did not alter outcome, compared to that of controls, at the 2-year follow-up.*
26	Vogrin M, Rupreht M, Dinevski D, et al.	2010	Effects of a platelet gel on early graft revascularization after anterior cruciate ligament reconstruction: a prospective, randomized, double-blind, clinical trial.	Eur Surg Res 2010; **45**(2): 77-85.	RCT	Anterior cruciate ligament (ACL) lesions	6 weeks	ND	***Local application of PRP improves early revascularisation of bone-ligament grafts after ACL reconstruction.***
27	Schepull T, Kvist J, Norrman H, et al.	2011	Autologous platelets have no effect on the healing of human achilles tendon ruptures: a randomized single-blind study.	Am J Sports Med 2011; **39**(1): 38-47.	RCT	Achilles' tendon rupture	1 year	30	*PRP is not useful in the treatment of ruptured Achilles' tendon.*
28	de Vos RJ, Weir A, Tol JL, et al.	2011	No effects of PRP on ultrasonographic tendon structure and neovascularisation in chronic midportion Achilles tendinopathy.	Br J Sports Med 2011; **45**(5): 387-92.	RCT	Achilles' tendinopathy	2 years	54	*Compared to placebo, PRP treatment does not increase tendon structure or neovascularisation of midportion Achilles' tendinopathy.*
29	de Jonge S, de Vos RJ, Weir A, et al.	2011	One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: a double-blind randomized placebo-controlled trial.	Am J Sports Med 2011; **39**(8): 1623-9.	RCT	Achilles' tendinopathy	1 year	54	*Compared to placebo, PRP is not related to improvements in pain and function in the treatment of midportion Achilles' tendinopathy.*
30	de Vos RJ, Weir A, van Schie HT, et al.	2010	Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial.	JAMA 2010; **303**(2): 144-9.	RCT	Achilles' tendinopathy	2 years	54	*Treatment with PRP does not appear to have significantly different effects from placebo.*
31	Sheth U, Simunovic N, Klein G, et al.	2012	Efficacy of autologous platelet-rich plasma use for orthopaedic indications: a meta-analysis.	J Bone Joint Surg Am 2012; **94**(4): 298-307.	Meta-analysis	Bone-tendon lesions	NA	NA	*Weak evidence of the utility of PRP in bone-tendon lesions.*
33	Ogundipe OK, Ugboko VI, Owotade FJ.	2011	Can autologous platelet-rich plasma gel enhance healing after surgical extraction of mandibular third molars?	J Oral Maxillofac Surg 2011; **69**(9): 2305-10.	RCT	Oral surgery	2 years	60	***Local application of PRP stimulates socket healing after third molar extraction.***
33	Ogundipe OK, Ugboko VI, Owotade FJ.	2011	Can autologous platelet-rich plasma gel enhance healing after surgical extraction of mandibular third molars?	J Oral Maxillofac Surg 2011; **69**(9): 2305-10.	RCT	Oral surgery	2 years	60	***Local application of PRP stimulates socket healing after third molar extraction.***

Legend RCT: randomised clinical trial; CCT: controlled clinical trial; NA: not applicable; ND: non available.

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Table II – Elements for evaluating the references.*(continued from previous page)*

Ref. #	Authors	Year	Title	Journal	Type of study	Subject	Follow up	N. of pts	Results (***Bold Italic: study with a positive result***; *Italic: study with a negative result*)
34	Marukawa E, Oshina H, Iino G, et al.	2011	Reduction of bone resorption by the application of platelet-rich plasma (PRP) in bone grafting of the alveolar cleft.	J Craniomaxillofac Surg 2011; **39**(4): 278-83.	CCT	Oral surgery	1 year	30	***Autologous cancellous bone grafting with PRP is an advantageous technique for alveolar bone grafting in patients with an alveolar cleft.***
35	Badr M, Coulthard P, Alissa R, Oliver R.	2010	The efficacy of platelet-rich plasma in grafted maxillae. A randomised clinical trial.	Eur J Oral Implantol 2010; **3**(3): 233-44.	RCT	Oral surgery	ND	22	*No appreciable effect seen when using PRP in autologous grafting of iliac crest bone in maxillary sinus augmentation.*
36	Del Fabbro M, Boggian C, Taschieri S.	2009	Immediate implant placement into fresh extraction sites with chronic periapical pathologic features combined with plasma rich in growth factors: preliminary results of single-cohort study.	J Oral Maxillofac Surg 2009; **67**(11): 2476-84.	CCT	Oral surgery	1 year	30	***The use of plasma, rich in growth factors during immediate implant placement can be considered safe and effective in alveolar treatment.***
37	Mooren RE, Merkx MA, Kessler PA, et al.	2010	Reconstruction of the mandible using preshaped 2.3-mm titanium plates, autogenous cortical bone plates, particulate cancellous bone, and platelet-rich plasma: a retrospective analysis of 20 patients.	J Oral Maxillofac Surg 2010; **68**(10): 2459-67.	CCT	Oral surgery	8 years	20	***Techniques involving PRP are easily used and related to osteoblastic proliferation and antimicrobial effect.***
38	Kotsovilis S, Markou N, Pepelassi E, Nikolidakis D.	2010	The adjunctive use of platelet-rich plasma in the therapy of periodontal intraosseous defects: a systematic review.	J Periodontal Res 2010; **45**(3): 428-43.	Review	Dentistry	NA	NA	***Positive*** and *negative* outcomes reported.
39	Esposito M, Grusovin MG, Rees J, et al.	2010	Interventions for replacing missing teeth: augmentation procedures of the maxillary sinus.	Cochrane Database Syst Rev 2010; (**3**): CD008397.	Systematic review	Dentistry	NA	NA	*No evidence that treatment with PRP and autologous bone or substitutes improves the clinical outcome of maxillary sinus augmentation procedures.*
40	Bae JH, Kim YK, Myung SK.	2011	Effects of platelet-rich plasma on sinus bone graft: meta-analysis.	J Periodontol 2011; **82**(5): 660-7.	Meta-analysis	Dentistry	NA	NA	***Sufficient evidence to support the use of PRP for bone formation on a sinus bone graft,*** *while there was no significant effect on implant survival.*
41	Marquez De Aracena Del Cid R, Montero De Espinosa Escoriaza I.	2009	Subconjunctival application of regenerative factor-rich plasma for the treatment of ocular alkali burns.	Eur J Ophthalmol 2009; **19**(6): 909-15.	RCT	Corneal burns by alkalis	35	NA	***Autologous PRP was an effective, economic treatment for ocular alkali burns.***
42	Alio JL, Abad M, Artola A, et al.	2007	Use of autologous platelet-rich plasma in the treatment of dormant corneal ulcers.	Ophthalmology 2007; **114**(7): 1286-1293 and 1281.	CCT	Corneal ulcers	38	NA	***Autologous PRP promoted healing of dormant corneal ulcers, even in cases of perforation, and reduced pain and inflammation.***

Legend RCT: randomised clinical trial; CCT: controlled clinical trial; NA: not applicable; ND: non available.