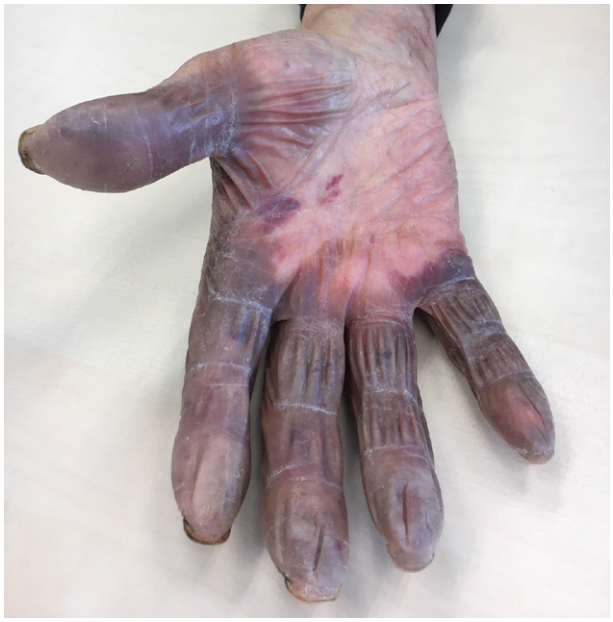
The Netherlands Journal of Medicine

PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE



Blue and purple spots on the hands: what is your diagnosis?

MARCH 2020, VOL. 78, NO. 02, ISSN 0300-2977

MacChain

The Netherlands Journal of Medicine

MISSION STATEMENT

To serve the needs of the physician to practice up-to-date medicine and to keep track of important issues in health care. To promote and to enhance clinical knowledge by publishing editorials, original articles, reviews, papers regarding specialty training, medical education and correspondence.

EDITORIAL INFORMATION

Editorial office
Erasmus MC, University Medical Center Rotterdam
Department of Internal Medicine
's-Gravendijkwal 230
3015 CE Rotterdam
The Netherlands
Tel.: +31 (0)10-703 59 54
Fax: +31 (0)10-703 32 68
E-mail: p.l.a.vandaele@erasmusmc.nl
http://mc.manuscriptcentral.com/nethjmed

CITED IN

Biosis database; embase/excerpta medica; index medicus (medline) science citation index, science citation index expanded, isi alerting services, medical documentation services, current contents/clinical medicine, PubMed.

ISSN: 0300-2977

Connecting Medical Initiatives

MacChain
PO Box 330
1960 AH Heemskerk
The Netherlands
Tel: +31 625056091
Email: info@macchain.nl
Web: www.macchain.nl

Contents

EDITORIAL

Zapping away hyperthyroidism

T.I.M. Korevaar

Department of Internal Medicine and Academic Center for Thyroid Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands. email: t.korevaar@erasmusmc.nl

Radiofrequency ablation (RFA) is a minimally invasive technique used to selectively induce tissue necrosis. Since the 1990s, RFA has been used for the treatment of benign and malignant disease, for example, by ablating an aberrant neurofibril bundle causing cardiac arrhythmia or ablation of hepatocellular carcinoma. Its first use for disease of the thyroid gland was described in 1998, when lymph node metastases from papillary thyroid cancer were successfully ablated. Nonetheless, it was not until 2006 that the first case series on its effect on primary thyroid nodules was described.[1] Since then, studies have shown a good efficacy of RFA for the treatment of benign (nodular) diseases of the thyroid gland. As such, the benefits of RFA outweigh potential harm, compared to conventional treatment modalities such as radioactive iodine therapy, antithyroid drugs, or (hemi)thyroidectomy. Combined with minimally invasive characteristics and preferred cosmetic outcome, these characteristics have paved the way for implementation of thyroid RFA in the Netherlands.[2]

In this issue of the journal, de Boer et al. report on their experience using RFA for the treatment of hyperactive thyroid nodules in 21 patients. Strengths of this study are the consecutive inclusion of patients over a three-year time period as well as the long follow-up ranging from 1.3 to 4.1 years. After 12 months, nodule volume decreased by about 60%, free thyroxine concentrations normalised in all patients, while thyroid-stimulating hormone concentrations normalised in 52%. Of those solely treated with RFA, biochemical control was achieved during the study period for all but one. Out of the total 21 patients, transient hyperthyroidism was seen in two patients, one of whom developed hypothyroidism requiring low-dose levothyroxine. Aside from transient mild local pain, no other adverse effects occurred. Pretty good statistics for a procedure that only took 3-16 minutes.

In parallel to the increased use of thyroid RFA, other minimally invasive techniques for thyroid ablation have also been introduced, such as ethanol/polidocanol ablation (mainly indicated for cystic lesions), microwave ablation, laser ablation, and high-frequency ultrasound ablation.[3] Comparative studies typically show that overall, RFA is equally as effective as other techniques, if not slightly more efficacious for volume reduction together with microwave ablation.[3-6] Worldwide, RFA currently is the most popular minimally invasive thyroid ablation technique because of the abundant experience for non-thyroidal indications and the wider availability of thyroid-related data including studies with a longer follow-up. However, as with most procedural interventions in medicine, the quality of the procedure depends on the experience and skillset of those who perform the procedure. From that perspective, it is reassuring to see that in the literature summary provided in the article by de Boer et al., the technique was practically equally as effective in the hands of the authors as in other centres. Although there is a low threshold for repeating the procedure, the slow (re)growth of thyroid nodules requires an even longer follow-up than performed in the current study in order to optimally quantify its true efficacy.

Interestingly, studies assessing the efficacy and safety of RFA for malignant thyroid disease are now beginning to make their way into the literature.[5] These studies show heterogeneous results as they typically include patients ineligible for surgical intervention or who had a small (typically < 1 cm) but histologically proven papillary microcarcinoma (an oncogenic entity for which a wait-and-see policy has become very plausible).[7] Nonetheless, initial studies show excellent volume reduction and implicate equal (recurrence rates), if not favourable outcomes (such as costs or quality of life) for RFA when compared to surgery in patients with either a primary or locally recurrent thyroid papillary (micro) carcinomas.[5,6,8,9] Thyroid RFA is a very welcome tool in the endocrinologists arsenal when battling thyroid nodules. When adequately studied, these techniques could prove valuable in reducing the potential (surgical) harms related to overdiagnosis of thyroid cancer caused by the incidental radiological detection of thyroid nodules.

REFERENCES

1. Kim YS, Rhim H, Tae K, Park DW, Kim ST. Radiofrequency ablation of benign cold thyroid nodules: initial clinical experience. Thyroid. 2006;16:361-7.
2. van Ginhoven TM, Massolt ET, Bijdevaate DC, Peeters RP, Burgers JW, Moelker A. [Radiofrequency ablation of a symptomatic benign thyroid nodule]. Ned Tijdschr Geneeskd. 2016;160:D202.
3. Feldkamp J, Grunwald F, Luster M, Lorenz K, Vorlander C, Fuhrer D. Non-Surgical and Non-Radioiodine Techniques for Ablation of Benign Thyroid Nodules: Consensus Statement and Recommendation. Exp Clin Endocrinol Diabetes. 2020. doi: 10.1055/a-1075-2025. [Epub ahead of print].
4. Cesareo R, Pacella CM, Pasqualini V, et al. Laser Ablation versus Radiofrequency Ablation for benign non-functioning thyroid nodules: Six-month results of a randomised, parallel, open-label, trial (LARA trial). Thyroid. 2020. doi: 10.1089/thy.2019.0660. [Epub ahead of print].
5. Choi Y, Jung SL. Efficacy and Safety of Thermal Ablation Techniques for the Treatment of Primary Papillary Thyroid Microcarcinoma: A Systematic Review and Meta-Analysis. Thyroid. 2020. doi: 10.1089/thy.2019.0707. [Epub ahead of print].
6. Tong M, Li S, Li Y, Li Y, Feng Y, Che Y. Efficacy and safety of radiofrequency, microwave and laser ablation for treating papillary thyroid microcarcinoma: a systematic review and meta-analysis. Int J Hyperthermia. 2019;36:1278-86.
7. Ito Y, Miyauchi A, Oda H. Low-risk papillary microcarcinoma of the thyroid: A review of active surveillance trials. Eur J Surg Oncol. 2018;44:307-15.
8. Zhang M, Tufano RP, Russell JO, et al. Ultrasound-Guided Radiofrequency Ablation Versus Surgery for Low-Risk Papillary Thyroid Microcarcinoma: Results of Over 5 Years' Follow-Up. Thyroid. 2020. doi.org/10.1089/thy.2019.0147. [Epub ahead of print].
9. Choi Y, Jung SL, Bae JS, et al. Comparison of efficacy and complications between radiofrequency ablation and repeat surgery in the treatment of locally recurrent thyroid cancers: a single-center propensity score matching study. Int J Hyperthermia. 2019;36:359-67.

REVIEW

Decreased bone mineral density and reproductive axis dysfunction: more than oestrogen

E.P. DeLoughery[1]*, M.L. Dow[2]

[1]Mayo Clinic School of Medicine, Mayo Clinic, [2]Department of Obstetrics and Gynecology, Mayo Clinic, Rochester Minnesota, USA. *Corresponding author: deloughery.emma@mayo.edu

ABSTRACT

Decreased bone mineral density (BMD) in oestrogen-deficient states has long been thought to be a direct outcome of the reduction in oestrogen. In physiologic and many pathologic hypo-oestrogenic states, oestrogen supplementation improves BMD. However, the relationship between oestrogen replacement and BMD is less clear in the case of reproductive axis dysfunction secondary to decreased caloric intake or increased energy expenditure, such as in female athletes or anorexia nervosa. This decrease in oestrogen is associated with decreased BMD, but oestrogen replacement in these states fails to conclusively improve BMD. This suggests that the decrease in BMD in these states is not driven solely by low oestrogen. Cortisol and other markers of inflammation may play a role in BMD reduction but further research is needed. What is clear is that increased caloric consumption and restoration of menses and the reproductive axis are essential to improving BMD, while pharmacologic therapy, including oestrogen replacement through hormone therapy or contraceptives, does not provide conclusive benefit.

KEY WORDS

Endocrinology, gynaecology, menopause, osteoporosis, women's health

INTRODUCTION

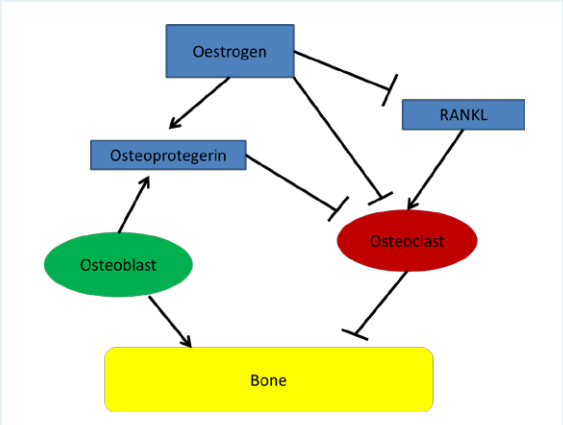
It is well-known that oestrogen-deficient states are a risk factor for osteoporosis. Bone mineral density (BMD) decreases dramatically in postmenopausal women and in women with Turner syndrome, Kallmann syndrome, and premature ovarian insufficiency.[1,2] Likewise, women with functional reproductive axis dysfunction (RAD), due, for example, to eating disorders or high-intensity sports activities, have a higher risk of low BMD. While the unifying factor in these states is the lack of oestrogen, the effect of oestrogen replacement on bone health is less than consistent. Oestrogen replacement improves BMD in many conditions, but there is a lack of a consistent relationship between oestrogen replacement and improved BMD in RAD. This suggests that there is another factor driving decreased BMD in women with RAD, and that the key to treatment is to look beyond oestrogen replacement.

Menopause

The most common and best-studied state of oestrogen deficiency is menopause, which is biochemically reflected in a decrease in oestrogen and an increase in follicle-stimulating hormone (FSH) and luteinizing hormone (LH).[3] Shortly after the onset of menopause, BMD decreases rapidly for the first 1-2 years after the final menses, with estimates of losses per year of 2.46% at the lumbar spine and 1.76% at the femoral neck.[4] This may lead to osteoporosis and bone fracture. Replacement of oestrogen in postmenopausal women is associated with increased BMD, with the 1996 Postmenopausal Estrogen/Progestin Interventions trial finding an average increase of 1.7% of BMD at the hip and a 3.5-5.0% average increase in spinal BMD for postmenopausal women taking oestrogen.[5] Risk factors for osteoporosis include advanced age, increased length of amenorrhea, family history of fracture, decreased dietary calcium intake, low body mass index, smoking, reduced physical activity, and increased alcohol intake.[4-6] The pathogenesis of this oestrogen deficiency-induced decrease in BMD appears to be loss of protective effects by oestrogen on the osteoblast/osteoclast ratio. Osteoblasts form new bone and are balanced by osteoclasts, which reabsorb bone. Osteoclasts are members of the monocyte-macrophage family and differentiate in

response to macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL), the former being expressed on osteoblasts and binding to its receptor on osteoclast precursors.[3] The RANKL receptor competes with the decoy receptor osteoprotegerin, which is secreted by osteoblasts to decrease osteoclast activation. Oestrogen induces osteoclast apoptosis and inhibits osteoclast development, perhaps by decreasing the responsiveness of osteoclast precursors to RANKL (figure 1). Oestrogen may also decrease production of RANKL and increase osteoprotegerin production.

Figure 1. *Mechanism of the effect of oestrogen on bone remodelling*



Arrows indicate stimulation while bars indicate inhibition
RANKL = receptor activator of nuclear factor kappa-B ligand

The immune system may also play a role in postmenopausal osteoporosis. T cells appear to be key regulators of the development and activity of osteoclasts and osteoblasts, although reduction in B-cell numbers may influence the development of osteoporosis.[3] Cytokines also have an effect, with a decrease in oestrogen correlating with an increase in pro-inflammatory cytokine production by bone marrow and bone cells, leading to increased bone resorption.[1] In particular, levels of interleukin (IL)-17, which stimulates osteoclast development while inhibiting osteoblasts, increase following menopause, and are inversely correlated with lumbar bone density.[3,7]

Turner & Kallmann Syndromes

Turning from physiologic to pathologic states of oestrogen deficiency, Turner syndrome is a form of hypergonadotropic hypogonadism with decreased oestrogen and increased FSH. When treated with adult doses of oestrogen, patients with Turner syndrome demonstrated significantly higher BMD than those who received lower doses of oestrogen.[8] When treatment was initiated prior to age 18, rate of BMD increase was 4.4% compared with 3.1% if initiation occurred after age 18. Patients with Turner syndrome who use continuous oestrogen replacement therapy demonstrate increased lumbar spine BMD, decreased risk of osteoporosis, and decreased vertebral compression fractures as compared with Turner syndrome patients not taking oestrogen replacement.[9] However, even with oestrogen replacement, individuals with Turner syndrome do not appear to reach peak bone mass, with one study finding an average z-score of -3.26 at the lumbar spine.[10] The pathogenesis of increased osteoporosis in Turner syndrome patients may be the increased development of osteoclasts, possibly due to increased FSH before oestrogen replacement.[11]

Unlike Turner syndrome, Kallmann syndrome is a form of hypogonadotropic hypogonadism, with low oestrogen and low FSH among female patients. Kallmann syndrome is also associated with reduced peak bone mass and increased risk for osteoporosis in the absence of hormone replacement, with one study finding an increased whole body z-score among patients who received appropriate hormone therapy as compared to those who did not (-0.3 vs. -1.7).[12] While hormone replacement therapy does appear to improve bone mass in these patients, lifelong therapy is needed.

One study compared hormone levels as well as bone mass and turnover in women diagnosed with Kallmann syndrome, Turner syndrome, or gonadal dysgenesis.[2] Patients with Kallmann syndrome had the lowest FSH and LH levels, as well as the lowest BMD, but there was no difference among the groups in oestradiol levels. This suggests a central role for oestrogen in mediating BMD; while other pituitary hormones vary among these three syndromes, oestrogen is deficient in all cases. In postmenopausal women, and in those with Turner syndrome and Kallmann syndrome, oestrogen deficiency is the common factor, and supplementation with oestrogen in all three cases increases BMD.

Reproductive axis dysfunction

Perhaps most similar to Kallmann syndrome is hypothalamic amenorrhea, in which dysfunction of the reproductive hypothalamic-pituitary-ovary axis occurs due to environmental or physiologic stress, such as decreased energy availability from decreased food intake or increased energy expenditure via increased physical activity. These states can be seen in patients with anorexia nervosa as well as female athletes, particularly in sports that value low body mass.[13,14] Female patients with any form of RAD experience decreased gonadotropin-releasing hormone (GnRH) secretion causing decreased LH and oestrogen levels.[15] Patients with anorexia nervosa in particular also experience decreases in dehydroepiandrosterone, testosterone, and insulin-like growth factor

(IGF-1) as well as an increase in cortisol.[14] These patients have low BMD and also a rapid decline in BMD, faster even than postmenopausal women, and are at an increased risk for fractures. Like postmenopausal women, they have low oestrogen, but patients with anorexia nervosa also have a variety of other hormonal imbalances that may influence their low BMD.

FEMALE ATHLETE TRIAD

A common and under-recognised condition, the female athlete triad is comprised of decreased energy intake, functional hypothalamic amenorrhea, and decreased bone density.[13] Like anorexia nervosa, decreased energy intake is likely the initiating factor, followed by hypothalamic suppression, and eventually decreased BMD. The combination of decreased energy and low oestrogen is thought to be responsible for the low BMD, though decreases in IGF-1 and leptin may also play a role.[13,16] While the energy intake restriction is often not as severe as in anorexia nervosa, the imbalance between energy intake and output in these female athletes is enough to suppress the hypothalamus.

Physical activity is usually thought of as beneficial to bone health, but in the female athlete triad too much activity can instigate and maintain bone fractures and low BMD. However, increased exercise even among amenorrhoeic athletes is associated with increased bone size, suggesting some protection against fracture.[13] The bones of amenorrhoeic athletes have a thinner cortical layer though, suggesting poor mineralisation, and thus weaker bone than that of eumenorrheic athletes. Type of activity influences BMD as well, with athletes participating in high-impact sports such as volleyball and soccer having no difference in BMD based on menstrual status, while athletes in low-impact sports such as swimming have decreased BMD if they are oligo/amenorrhoeic. Therefore, weight-bearing or resistance exercise may be of benefit in improving or retaining BMD.[13,14] The key, however, is to combine the bone-improving effects of physical activity with an internal environment most conducive to the formation and maintenance of healthy bone. To many, the essential component of that environment is oestrogen with its apoptotic effect on osteoclasts, thereby improving the osteoblast to osteoclast ratio.

TREATMENT

A popular treatment for individuals with RAD or otherwise abnormal menses is use of oral contraceptives (OCP).[14,17] However, a consensus on the effects of OCP on bone health does not exist, with some studies finding an increase in BMD and others finding no effect.[13,18-20] A 2017 meta-analysis found no evidence to support use of hormonal therapy to improve bone health in this population, with a weighted mean difference of 0.02 g/cm^2 between total body BMD among patients receiving hormonal therapy and controls.[21] Likewise, the effect of treatment with oestrogen or androgens in women with RAD also appears unclear, while use of recombinant IGF-1, recombinant leptin, teriparatide, and denosumab requires more investigation.[13] In fact, high doses of oestrogen may inhibit secretion of IGF-1 and testosterone, which may decrease BMD.[14,15] The best treatment to increase bone density in patients with RAD appears to be increased caloric intake with associated weight gain and resumption of menses.[13,15,22] While resumption of menses would indicate increased oestrogen, replacement of oestrogen alone does not correlate with increased BMD. Whereas in postmenopausal women, exogenous oestrogens are able to improve bone mass, the benefits among RAD patients are debatable.[15] This suggests another player in the genesis of decreased BMD in these patients. That player may be inflammation.

Inflammation

Like oestrogen deficiency, increased levels of glucocorticoids are associated with increased risk of osteoporosis. Glucocorticoids inhibit periosteal cell proliferation, decreasing the number of osteoblasts and thus inhibiting bone formation.[23,24] Increased urinary free cortisol excretion in healthy young women is associated with decreased BMD.[25] Patients with RAD, including that induced by exercise and anorexia nervosa, have elevated cortisol levels.[26,27] High levels of cortisol are associated with decreased LH secretion among amenorrhoeic-exercising women as compared with eumenorrheic women, as well as with increased markers of bone resorption and decreased markers of bone formation.[28] Inflammation is directly linked to cortisol as high levels of inflammatory cytokines cause a rise in adrenocorticotropic hormone (ACTH) and cortisol.[29] Patients with anorexia nervosa have increased levels of inflammatory cytokines, particularly tumour necrosis factor (TNF)-alpha, IL-1β, and IL-6.[30] States of high inflammation, such as autoimmune disorders, are associated with increased bone loss.[3] It is possible that the inflammatory state in RAD patients is induced by oestrogen deficiency, as can be seen with increased levels of pro-inflammatory cytokines in postmenopausal women.[1] However, that the low BMD remains even when oestrogen is replaced in RAD patients argues against this and suggests that another factor induces the inflammation. In general, exercise appears to reduce markers of inflammation in several populations.[31,32] However, in highly-trained athletes cortisol levels are often mildly elevated, as are

levels of the pro-inflammatory cytokine IL-6, which in turn increases levels of ACTH and cortisol, resulting in a vicious cycle of increased inflammation and cortisol production.[24] IL-6 stimulates development of osteoclasts, thereby directly contributing to decreased BMD. In fact, elevated cortisol levels, as are found in both exercise- and anorexia nervosa-induced RAD, may be the initiating factor of the condition. Corticotropin-releasing hormone, the upstream hormone in the pathway containing cortisol and ACTH, suppresses the release of GnRH and consequently of LH, FSH, and oestrogen.[24]

Leptin

Another possible mediator of decreased BMD in RAD patients is leptin. Produced primarily by adipocytes, leptin affects food intake, with other roles currently under investigation. Leptin levels reflect the amount of energy stored in fat, and decreased energy availability leads to decreased leptin levels.[33] Leptin levels alone do not correlate with BMD and thus may influence bone density indirectly.[34] In a randomised controlled trial of patients with hypothalamic amenorrhea, administration of recombinant leptin resulted in resumption of menses, increased oestradiol and progesterone levels, and increased osteocalcin, a marker of bone formation, although there was no difference between treatment groups in BMD.[33] Another study found treatment with recombinant leptin decreased RANKL and increased osteoprotegerin levels.[34]

CONCLUSION

When discussing improvement of BMD and prevention of osteoporosis, replacement of oestrogen should be part of the conversation for women who are postmenopausal, or have Turner or Kallmann syndromes. If a patient's reproductive axis is suppressed due to an external cause, however, the current research does not support use of exogenous oestrogens, or indeed any pharmacologic therapy, for the restoration of BMD. Instead, addressing the underlying cause, increasing caloric intake and resumption of menses appears to be the current gold standard. This discrepancy between treatments and results in different oestrogen-deficient states suggests that oestrogen alone is not the cause of the decreased BMD in patients with suppressed reproductive axis. Strong contenders for other potential mediators include cortisol and a general state of inflammation, as well as leptin. Further study is needed to more clearly define the cause of decreased BMD in patients whose reproductive axis is suppressed.

DISCLOSURES

The authors declare no conflicts of interest. No funding or financial support was received.

REFERENCES

1. Brincat SD, Borg M, Camilleri G, Calleja-Agius J. The role of cytokines in postmenopausal osteoporosis. Minerva Ginecol. 2014;66:391-407.
2. Castelo-Branco C, Leon M, Duran M, Balasch J. Follicle-stimulating hormone does not directly regulate bone mass in human beings: evidence from nature. Fertil Steril. 2008;90:2211-6.
3. Faienza MF, Ventura A, Marzano F, Cavallo L. Postmenopausal osteoporosis: the role of immune system cells. Clin Dev Immunol. 2013;2013:575936.
4. Cauley JA. Estrogen and bone health in men and women. Steroids. 2015;99(Pt A):11-5.
5. Effects of hormone therapy on bone mineral density: results from the postmenopausal estrogen/progestin interventions (PEPI) trial. The Writing Group for the PEPI. Jama. 1996;276:1389-96.
6. Ziller M, Herwig J, Ziller V, Kauka A, Kostev K, Hadji P. Effects of a low-dose oral estrogen only treatment on bone mineral density and quantitative ultrasonometry in postmenopausal women. Gynecol Endocrinol. 2012;28:1002-5.
7. Molnar I, Bohaty I, Somogyine-Vari E. High prevalence of increased interleukin-17A serum levels in postmenopausal estrogen deficiency. Menopause. 2014;21:749-52.
8. Kodama M, Komura H, Kodama T, Nishio Y, Kimura T. Estrogen therapy initiated at an early age increases bone mineral density in Turner syndrome patients. Endocr J. 2012;59:153-9.
9. Hanton L, Axelrod L, Bakalov V, Bondy CA. The importance of estrogen replacement in young women with Turner syndrome. J Womens Health (Larchmt). 2003;12:971-7.
10. Lanes R, Gunczler P, Esaa S, Martinis R, Villaroel O, Weisinger JR. Decreased bone mass despite long-term estrogen replacement therapy in young women with Turner's syndrome and previously normal bone density. Fertil Steril. 1999;72:896-9.
11. Faienza MF, Brunetti G, Ventura A, et al. Mechanisms of enhanced osteoclastogenesis in girls and young women with Turner's Syndrome. Bone. 2015;81:228-36.
12. Laitinen EM, Hero M, Vaaralahti K, Tommiska J, Raivio T. Bone mineral density, body composition and bone turnover in patients with congenital hypogonadotropic hypogonadism. Int J Androl. 2012;35:534-40.
13. Mallinson RJ, De Souza MJ. Current perspectives on the etiology and manifestation of the "silent" component of the Female Athlete Triad. Int J Womens Health. 2014;6:451-67.
14. Jagielska GW, Przedlacki J, Bartoszewicz Z, Racicka E. Bone mineralization disorders as a complication of anorexia nervosa - etiology, prevalence, course and treatment. Psychiatr Pol. 2016;50:509-20.
15. Foo JP, Hamnvik OP, Mantzoros CS. Optimizing bone health in anorexia nervosa and hypothalamic amenorrhea: new trials and tribulations. Metabolism. 2012;61:899-905.
16. Grinspoon S, Miller K, Coyle C, et al. Severity of osteopenia in estrogen-deficient women with anorexia nervosa and hypothalamic amenorrhea. J Clin Endocrinol Metab. 1999;84(6):2049-55.
17. Hergenroeder AC. Bone mineralization, hypothalamic amenorrhea, and sex steroid therapy in female adolescents and young adults. J Pediatr. 1995;126(5 Pt 1):683-9.

18. Lloyd T, Taylor DS, Lin HM, Matthews AE, Eggli DF, Legro RS. Oral contraceptive use by teenage women does not affect peak bone mass: a longitudinal study. Fertil Steril. 2000;74:734-8.
19. Sultana S, Choudhury S, Choudhury SA. Effect of combined oral contraceptives on bone mineral density in pre and postmenopausal women. Mymensingh Med J. 2002;11:12-4.
20. Martins SL, Curtis KM, Glasier AF. Combined hormonal contraception and bone health: a systematic review. Contraception. 2006;73:445-69.
21. Altayar O, Al Nofal A, Carranza Leon BG, Prokop LJ, Wang Z, Murad MH. Treatments to Prevent Bone Loss in Functional Hypothalamic Amenorrhea: A Systematic Review and Meta-Analysis. J Endocr Soc. 2017;1:500-11.
22. Vescovi JD, Jamal SA, De Souza MJ. Strategies to reverse bone loss in women with functional hypothalamic amenorrhea: a systematic review of the literature. Osteoporos Int. 2008;19:465-78.
23. Chyun YS, Kream BE, Raisz LG. Cortisol decreases bone formation by inhibiting periosteal cell proliferation. Endocrinology. 1984;114:477-80.
24. Mastorakos G, Pavlatou M, Diamanti-Kandarakis E, Chrousos GP. Exercise and the stress system. Hormones (Athens). 2005;4:73-89.
25. Bedford JL, Barr SI. The relationship between 24-h urinary cortisol and bone in healthy young women. Int J Behav Med. 2010;17:207-15.
26. Lawson EA, Donoho D, Miller KK, et al. Hypercortisolemia is associated with severity of bone loss and depression in hypothalamic amenorrhea and anorexia nervosa. J Clin Endocrinol Metab. 2009;94:4710-6.
27. Ding JH, Sheckter CB, Drinkwater BL, Soules MR, Bremner WJ. High serum cortisol levels in exercise-associated amenorrhea. Ann Intern Med. 1988;108:530-4.
28. Ackerman KE, Patel KT, Guereca G, Pierce L, Herzog DB, Misra M. Cortisol secretory parameters in young exercisers in relation to LH secretion and bone parameters. Clin Endocrinol (Oxf). 2013;78:114-9.
29. Straub RH, Cutolo M. Glucocorticoids and chronic inflammation. Rheumatology (Oxford). 2016;55(suppl 2):ii6-ii14.
30. Solmi M, Veronese N, Favaro A, et al. Inflammatory cytokines and anorexia nervosa: A meta-analysis of cross-sectional and longitudinal studies. Psychoneuroendocrinology. 2015;51:237-52.
31. Neefkes-Zonneveld CR, Bakkum AJ, Bishop NC, van Tulder MW, Janssen TW. Effect of long-term physical activity and acute exercise on markers of systemic inflammation in persons with chronic spinal cord injury: a systematic review. Arch Phys Med Rehabil. 2015;96:30-42.
32. Hayashino Y, Jackson JL, Hirata T, et al. Effects of exercise on C-reactive protein, inflammatory cytokine and adipokine in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. Metabolism. 2014;63:431-40.
33. Chou SH, Chamberland JP, Liu X, et al. Leptin is an effective treatment for hypothalamic amenorrhea. Proc Natl Acad Sci U S A. 2011;108:6585-90.
34. Foo JP, Polyzos SA, Anastasilakis AD, Chou S, Mantzoros CS. The effect of leptin replacement on parathyroid hormone, RANKL-osteoprotegerin axis, and Wnt inhibitors in young women with hypothalamic amenorrhea. J Clin Endocrinol Metab. 2014;99:E2252-8.

ORIGINAL ARTICLE

Measurement of anti-TSH receptor antibodies: what is the correct cut-off value?

M.A. Smit[1], C.M.J. van Kinschot[2], J. van der Linden[2], C. van Noord[2], S. Kos[1]*

Departments of [1]Clinical Chemistry, [2]Internal Medicine, Maasstad Hospital, Rotterdam, the Netherlands. *Corresponding author: koss@maasstadziekenhuis.nl

ABSTRACT

Background: Autoantibodies against the thyroid stimulating hormone receptor, thyrotropin receptor autoantibodies (TRAb) are diagnostic for Graves' disease and can be measured by different methods. As antibody concentrations are not comparable between methods, appropriate cut-off values need to be established for every single method. For a third-generation TRAb assay (Phadia, Thermofisher), the manufacturer determined the cut-off value in a study population consisting of Graves' disease (both newly diagnosed and patients under treatment) and non-Graves' disease patients. The aim of this study was to verify whether this cut-off value holds true in our population.

Methods: Retrospective analysis was performed on TRAb measurements collected over a period of six months from all patients referred for TRAb testing. For our study, we included patients that were newly diagnosed with hyperthyroidism including Graves' disease, multinodular goitre, toxic adenoma, and thyroiditis. Furthermore, we included Graves' patients that were under treatment at the time of TRAb measurement.

Results: Whereas all patients with Graves' disease had positive TRAb, few patients with multinodular goitre, toxic adenoma, and thyroiditis scored positive for TRAb. ROC curve analysis revealed a cut-off value of 4.5 IU/l (compared to 3.3 IU/l established by the manufacturer). Newly diagnosed Graves' patients had higher TRAb concentrations compared to patients under treatment.

Conclusion: The cut-off value of this immunoassay should probably be set higher in untreated Graves' patients than proposed by the manufacturer as the cut-off value should be determined in a study population excluding Graves' patients under treatment. The overall clinical picture remains crucial in the diagnosis of Graves' disease.

KEYWORDS

Cut-off value, Graves' disease, TRAb, TSH receptor antibody

INTRODUCTION

Graves' disease is a frequent cause of hyperthyroidism which can be accompanied by goitre and/or eye disease (orbitopathy).[1] The disease is caused by thyrotropin receptor autoantibodies (TRAb). These autoantibodies, present in almost 100% of patients with Graves' disease, can stimulate the thyroid stimulating hormone (TSH) receptor and thereby cause hyperthyroidism.[2] Detection of TRAb is important in the discrimination of Graves' disease from other causes of hyperthyroidism. In addition to stimulating autoantibodies, certain TRAbs exhibit blocking or neutralising activity. Blocking TRAbs are less common but important to measure, as these antibodies can cause hypothyroidism,[3] which can be accompanied by orbitopathy[4] and myxoedema.[5] TRAbs are present in 1-2% of healthy people and in 6-60% patients with Hashimoto disease.[6] As TRAbs are diagnostic for Graves' disease,[7] guidelines recommend measurement of TRAbs in several patient groups. First, in patients with hyperthyroidism to determine the aetiology of thyrotoxicosis (the American Thyroid Association (ATA) Guideline[8] and the Dutch Internist Association (NIV) guideline).[9] Second, as TRAbs can cross the placenta and cause foetal hyperthyroidism, measurement is recommended in pregnant women that have or have had Graves' disease (Dutch guideline of Obstetrics and Gynaecology, NIV guideline and International guideline for management of thyroid dysfunction during pregnancy).[9-11]

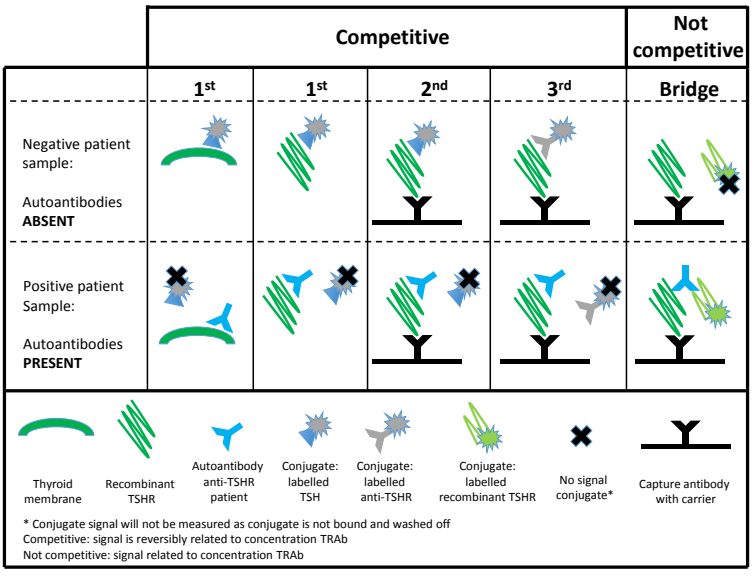
There are different types of assays that can measure TRAb levels: bioassays and bridge immunoassays specifically measure stimulating antibodies but not blocking

antibodies, whereas competitive immunoassays cannot discriminate between stimulating and blocking antibodies. The latter are the most widely used within laboratories. Of note, even within this assay group, several rounds of optimisation in assay design have taken place, resulting in different assay generations that are incomparable in their measurement (for a complete description and overview see figure 1). Furthermore, as TRAb assays are not standardised (i.e., may be calibrated against different internationally recognised reference materials and/or differ methodologically), concentrations from assays of different companies are not easily comparable.[12,13] Overall, due to several reasons, different assays are not comparable and therefore, clinically relevant cut-off values must be established for every method separately.

Recently, a third-generation fully-automated competitive immunoassay (Thermofisher, Phadia, Sweden) was introduced in our laboratory. This assay has been calibrated against the most recent reference standard (World Health Organization (WHO) standard 08/204). The manufacturer determined the cut-off value in an internal study (n = 400, including patients with Graves' disease, Hashimoto's thyroiditis, non-autoimmune thyroid diseases, and a variety of non-thyroid diseases).[14] The group of Graves' patients consisted of a mixture of patients that were newly diagnosed and patients that were under treatment. However, upon treatment with anti-thyroid drugs or surgery, TRAb concentration can decline. For example, after one year of anti-thyroid drug treatment, approximately 60% of the treated patients are negative for TRAb measurement.[14] Treatment with RAI ultimately also leads to a decrease in TRAb concentration, although RAI can lead, in the first year, to an initial increase.[15,16]

In this study, the aim was to verify that the cut-off value established by the manufacturer also holds true in our population. Additionally, the aim was to study the influence of including treated Graves' disease patients in the determination of the cut-off value, since TRAb measurement is primarily used for diagnostic purpose, and the cut-off value could be influenced by patients treated.

Figure 1. *Overview of different generations of competitive immunoassays and the bridge assay. In a first-generation immunoassay, autoantibodies in the patient's serum compete with either porcine thyroid membrane or recombinant TSH receptor. Important to note, is that this assay is in solution, making it impossible to wash. In a second-generation assay, autoantibodies in patient's serum can bind the recombinant TSH receptor bound to a capture membrane. The autoantibodies compete with recombinantly labelled TSH. The difference in a third-generation assay is that the autoantibodies compete with a recombinant monoclonal antibody against the TSH receptor. In a bridge assay, a chimeric recombinant TSH receptor that contains only the binding part where the stimulating autoantibodies can bind, is bound to a capture membrane. A labelled recombinant TSH receptor can be used as detection.*



TRAb = autoantibodies against TSH receptor; TSHR = TSH receptor

MATERIALS AND METHODS

Patients

Samples used for retrospective analyses were from all patients referred for TRAb testing from February to April 2018 and November 2018 to January 2019 from two hospitals (Maasstad Hospital, Rotterdam and Spijkenisse Medical Centre, Spijkenisse, both in the Netherlands). For our study, we included patients that were newly diagnosed for the most frequent causes of hyperthyroidism and who fulfilled the following criteria: age > 18 years, not pregnant, seen by an internist, and no medical history of Graves' disease. These 61 patients consisted of 28 patients with Graves' disease, 15 with multinodular goitre or toxic adenoma, and 18 with thyroiditis. In addition, we included Graves' patients that were under treatment with antithyroid drugs at the time of TRAb measurement. Treatment was either monotherapy thiamazole or in combination with thyroid hormone (the so-called block and replace therapy). Graves' disease was diagnosed according to both the NIV guideline[9] and the comparable American guideline.[8] More specifically, the diagnosis was based on the presence of (subclinical) hyperthyroidism, with clinical context including presence of orbitopathy and diffuse uptake as seen with 99mTc scintigraphy, or diffuse enlargement of the thyroid by ultrasound. The criteria for Graves' disease were presence of (subclinical) hyperthyroidism, clinical symptoms fitting Graves' disease, and positive TRAb. Of note, as the aim of this study was to determine the cut-off value for the TRAb assay; 6/7 patients with TRAb concentrations below 6 IU/l had, in addition to (subclinical) hyperthyroidism, scintigraphy results resembling Graves' disease. In one patient with TRAb concentration of 4.5 IU/l, radiography was not performed. This patient was diagnosed with Graves' disease because clinical follow-up of treatment matched Graves' disease, the typical age (30 years), and absence of palpable nodes in the thyroid. The study was approved by the local medical ethical committee.

Methods

Samples were measured with a third-generation competitive immunoassay: EliA-anti-TSH-R well (Thermofisher Scientific, Phadia, Sweden). The method is based on the competition of the autoantibody with a monoclonal antibody against the TSH receptor labelled with beta-galactosidase. This method is standardised against the most recent WHO standard 08/204. According to the manufacturer, the interval of 2.9-3.3 IU/l is referred to as grey zone area and a concentration above 3.3 IU/l is considered positive. The total coefficient of variance (CV) was established by determination of the within run and between run CV of three samples with different concentrations of TRAb. The within run resembles the analytical variation based on multiple measurements of the same sample within one run. The between run variation resembles the analytical variation of the same sample measured on different days. Between run and within run CV were calculated by duplicate measurements of the same sample in 10 different runs on five different days using a common laboratory procedure protocol for the determination of random error using the EP evaluator version 12 software (Data Innovations LLC, South Burlington, VT, USA).

Statistical analysis

The distribution of TRAb results showed non-Gaussian distribution, therefore the results are presented as median and interquartile range. In order to determine the optimal cut-off value, receiver operating characteristics (ROC) curve analysis was performed with EP evaluator version 12 software. This analysis revealed the percent efficiency: the percent of all results that are classified correctly (the true positives and true negatives). The optimal cut-off value was determined as the cut-off value with maximal percent efficiency.

RESULTS

Description of the population

Characteristics of the different study groups are summarised in table 1. The whole patient group consisted of more women (85%, n = 63) than men (15%, n = 11). All patients with Graves' disease were women.

Patients with Graves' disease presented with hyperthyroidism, as observed by median concentrations of TSH and FT4, whereas the TSH and FT4 median concentrations in patients treated for Graves' disease resembled normal thyroid function tests. Almost all (14/15) newly diagnosed Graves' patients had either ultrasound or thyroid scintigraphy results compatible with Graves' disease. Of note, one patient did not show signs of Graves' disease on ultrasound, and scintigraphy was not performed. Although Graves' disease is not always detected by ultrasound, it is the overall clinical picture that the clinician considers to determine a diagnosis; this patient was diagnosed with Graves' disease because of the presence of orbitopathy. As expected, the median concentration of TRAb was higher in newly diagnosed patients compared to patients under treatment (median TRAb 9.9 IU/l compared to 4.8 IU/l, respectively).

Patients with multinodular goitre and thyroiditis presented with subclinical hyperthyroidism (median TSH for both < 0.06 mU/l, median FT4 in normal range). Both groups had negative (< 2.9, based on cut-off value of manufacturer)

Table 1. *Characteristics of the study population*

	Group I Graves (untreated)	Group II Graves (under treatment)	Group III MNG/toxic adenoma	Group IV Thyroiditis
n	**28**	**13**	**15**	**18**
Gender (F/M)	28/0	9/4	12/3	14/4
Age (years)				
Mean ± SD	43 ± 17	40 ± 20	62 ± 20	49 ± 19
Min-max	18-77	18-82	25-88	29-92
Median	40	33	65	47
1st-3rd quartile	31-57	23-52	45-79	33-64
Laboratory evaluation				
TSH (0.4-4.0 mU/l)				
Mean ± SD	0.06 ± 0.26	6.4 ± 18.4	0.6 ± 1.3	7.2 ± 23.6
Min-max	< 0.01-1.4	< 0.01-67.5	< 0.01-3.8	< 0.01-18.4
Median	< 0.01	1.1	0.04	< 0.01
1st-3rd quartile	< 0.01-< 0.01	< 0.01-1.8	0.02-0.36	< 0.01-0.33
FT4 (10-24 pmol/l)				
Mean ± SD	38.3 ± 15.3	20.9 ± 13.6	21.3 ± 12	33.1 ± 33.1
Min-max	12.4-75.4	10.1-60.6	11.3-43	3.6-99
Median	35.0	17.3	13.8	16.2
1st-3rd quartile	28-47	13.6-23.2	12.8-31.2	13.0-36.1
TRAb (< 3.3 IU/l)				
Mean ± SD	13 ± 11.5	12.2 ± 18.6	3.2 ± 1.5	2.6 ± 0.7
Min-max	3.4-52	1.7-68	1.5-6.1	1.5-3.7
Median	9.9	4.8	2.8	2.7
1st-3rd quartile	6.4-13.8	3.7-6.9	2.3-3.7	2.3-3.1
Clinical evaluation				
Thyroid status				
Hyperthyroidism	22	4	5	9
Subclinical hyperthyroidism	5	2	10	5
Euthyroidism	1	4	0	1
(Subclinical) hypothyroidism	0	3	0	3
Radiology resembling Graves' disease				
Echography positive*	1		0	0
Echography negative	1		4	6
Scintigraphy positive**	13		0	1
Scintigraphy negative	0		9	4
Not performed	13		2	7
Orbitopathy				
Present	6		1	0
Absent	22		14	18

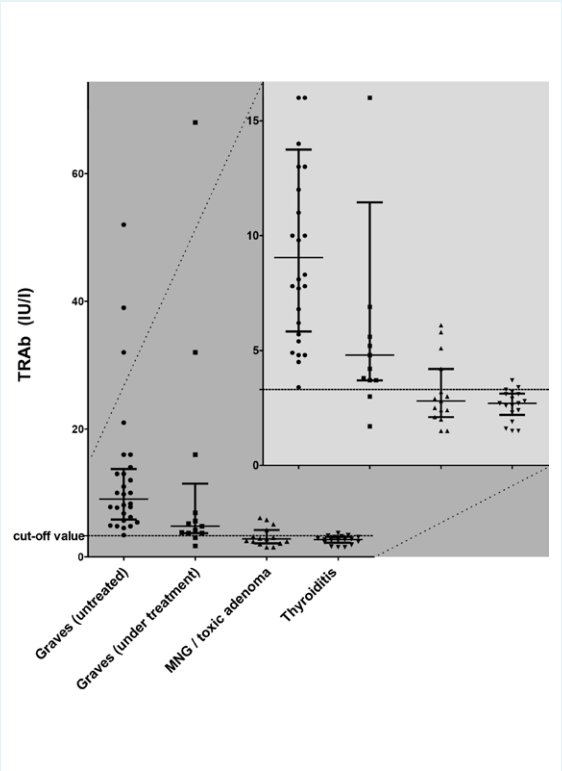
* Diffuse enlargement of the thyroid by ultrasound
** Diffuse uptake seen on 99mTc scintigraphy
F = female; FT4 = free thyroid hormone; M = male; Min = minimum; Max = maximum; MNG = multinodular goitre; SD = standard deviation; TRAb = thyrotropin receptor autoantibodies; TSH = thyroid stimulating hormone

median TRAb concentrations. When radiology was performed, almost all (22/23) had ultrasound or scintigraphy results not resembling Graves' disease. The patient that had a scintigraphy resembling Graves' disease had diffuse thyroid uptake of radioactive iodine. This image is typical for Graves' disease, but can also be seen for patients with thyroiditis. This patient had typical clinical symptoms resembling thyroiditis as within one month, the patient developed hypothyroidism. After half a year, the patient was symptom free with normal thyroid function.

Random error: coefficient of variance

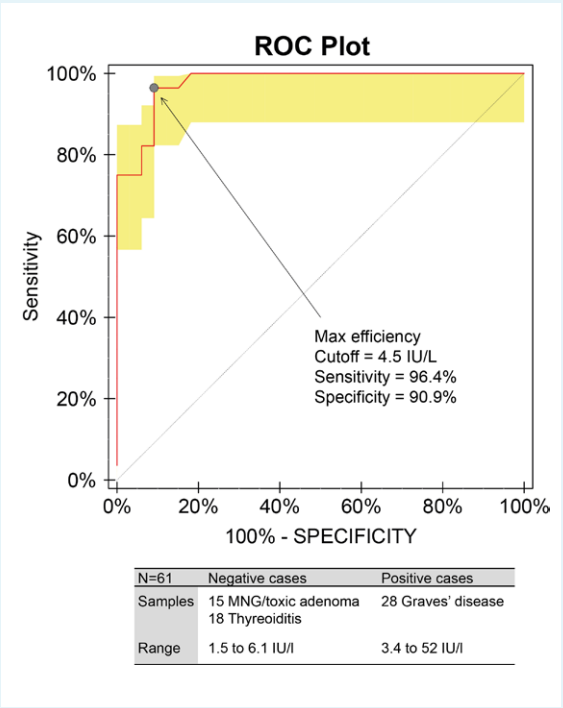
We determined the within run, between run, and total CV (Supplementary table 1). The CV increases with decreasing antibody concentrations, and is relatively high in the lower concentrations. The sample with a TRAb concentration of

Figure 2. *TRAb concentration in the different patient groups*
Dot plot depicting TRAb concentrations within each patient group. Threshold value represents the value as presented by the manufacturer (3.3 IU/l).



MNG = multinodular goitre; N = number of patients; TRAb = thyrotropin receptor autoantibodies

Figure 3. *ROC curve analysis*
ROC curve analysis of 61 patients consisting of 28 patients with newly diagnosed Graves' disease, 15 patients with multinodular goitre and/or toxic adenoma, and 18 patients with thyroiditis. At a threshold value of 4.5 IU/l, the sensitivity is 96.4% and the specificity is 90.9%.



MNG = multinodular goitre; N = number of patients; ROC = receiver operating characteristic

2.2 IU/l had a total CV of 26.4%, whereas at 9.6 IU/l, the total CV was 16.7%. According to the manufacturer, the CV at the cut-off value 3.3 IU/l is 15%, but at 2.9 IU/l > 20%.

TRAb assay: grey zone and cut-off value

We analysed the TRAb concentration in all different patient groups (figure 2). When the manufacturer's cut-off value of 3.3 IU/l was used as positive for TRAb, all Graves' disease patients were positive for TRAb. Of all Graves' disease patients that received treatment (mostly block and replace treatment), 85% (n = 11) scored positive for antibodies. Median value of TRAb concentration of multinodular goitre and toxic adenoma patients was negative, 2.8 IU/l. However, four patients (27%) scored positive with TRAb

Table 2. *Number of patients in the different grey zones*

	TRAb concentration range	
	2.9-3.3 IU/l grey zone: manufacturer (# patients)	3.3-4.5 IU/l grey zone: our analysis (# patients)
Non-Graves' disease: MNG, toxic adenoma and thyroiditis	7	4
Newly diagnosed Graves' disease	0	1
Total	**7**	**5**

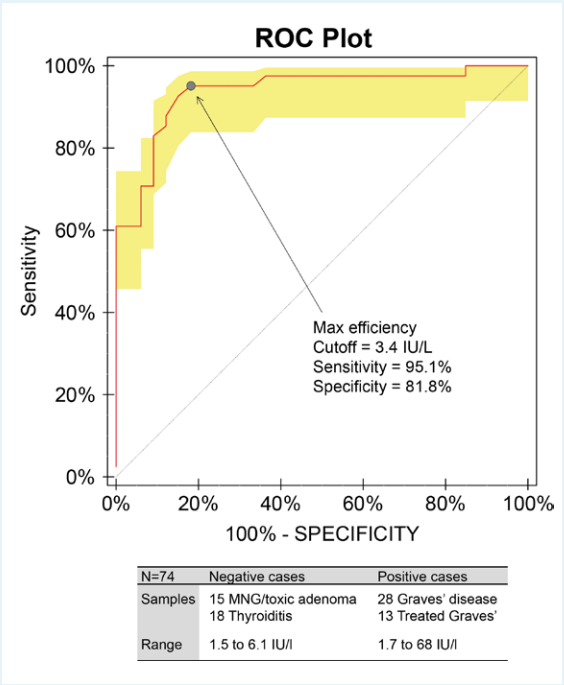
MNG = toxic multinodular goitre; TRAb = thyrotropin receptor autoantibodies

Table 3. *Sensitivity and specificity of TRAb measurement at different cut-off values*

	Negative TRAb (# patients)	Positive TRAb (# patients)	Sensitivity	Specificity
TRAb cut-off value 2.9 IU/l			**100**	**58**
Non-Graves' disease*	19	14		
Graves' disease**	0	28		
TRAb cut-off value 3.3 IU/l			**100**	**79**
Non-Graves' disease*	26	7		
Graves' disease**	0	28		
TRAb cut-off value 4.5 IU/l			**96**	**91**
Non-Graves' disease*	30	3		
Graves' disease**	1	27		

* MNG, toxic adenoma or thyroiditis
** newly diagnosed Graves' disease
TRAb = thyrotropin receptor autoantibodies

Figure 4. *ROC curve analysis including patients under treatment.*
ROC curve analysis of 74 patients where Graves' patients that were under treatment at the time of TRAb measurement were included in the positive cases. The study population consisted of 28 patients with newly diagnosed Graves' disease, 13 patients with Graves' disease under treatment, 15 patients with multinodular goitre and/or toxic adenoma, and 18 patients with thyroiditis.



MNG = multinodular goitre; N = number of patients;
ROC = receiver operating characteristic

concentration ranging from 4.2-6.1 IU/l. One possibility might be that these patients have a combination of multinodular goitre and Graves' disease. Of all thyroiditis patients, the majority (16/18) had negative TRAb, whereas 11% (2/18) scored positive for antibodies. However, TRAb concentration was relatively low (TRAb concentration of 3.4 and 3.7 IU/l, respectively).

All data from the patient groups, excluding Graves' disease patients under treatment, were plotted in an ROC curve. ROC curve analysis showed that the most accurate threshold value for this cohort is 4.5 IU/l (figure 3). This cut-off value is higher than the threshold value proposed by the manufacturer, implying that the upper limit of the grey zone should be 4.5 IU/l instead of 3.3 IU/l. Of note, none of the Graves' patients were in the grey zone defined by the manufacturer, whereas 1/5 patients had Graves' disease in the newly defined grey zone (table 2).

We determined the sensitivity and specificity of the different cut-off values: 2.9 IU/l (lower limit of the grey zone proposed by the manufacturer), 3.3 IU/l (cut-off for positivity of the manufacturer), and 4.5 IU/l (cut-off for positivity based on our study population). Applying a cut-off value of 4.5 resulted in a sensitivity of 96% and specificity of 91%, compared to a sensitivity of 100% and a specificity of 79% when the cut-off value of 3.3 IU/l was used (table 3).

The influence of including patients under treatment on the cut-off value was determined by adding patients under treatment to the group of newly diagnosed Graves' patients. This revealed a cut-off value of 3.4 IU/l, comparable to the one established by the manufacturer (figure 4).

One important issue is that, four patients with multinodular goitre, had positive TRAb concentrations

which could be explained by the co-existence of Graves' disease. TRAb concentrations of these patients ranged from 4.2 to 6.1 IU/l. This is in agreement with data from another study, where several patients with MNG scored positive for TRAb.[17] To exclude that the cut-off value was influenced by these four patients, we repeated the ROC curve analysis without these four patients. This did not change the cut-off value of 4.5 IU/l (Supplementary figure 1A). Analysis of the data with these four patients in the Graves' disease group however, revealed a cut-off value of 4.2 IU/l (Supplementary figure 1B). This cut-off value is still higher than that established by the manufacturer. Overall, the grey zone area and the cut-off value based on newly diagnosed Graves' patients is higher in this patient cohort than the one from the manufacturer.

DISCUSSION

As shown in this study, TRAb is clearly increased in untreated Graves' patients, lower in treated Graves' patients, and marginally increased or even absent in patients with multinodular goitre and thyroiditis. Our analysis revealed that excluding patients under treatment results in a higher cut-off value for TRAb positivity (4.5 IU/l compared to 3.3 IU/l, as recommended by the manufacturer). As TRAb concentrations are primarily used to establish the diagnosis of Graves' disease, extending the grey zone to 4.5 IU/l is probably more appropriate and will more accurately discriminate between Graves' disease and non-Graves' disease causes of hyperthyroidism.

Reference values are usually established by measuring samples in a group of ostensibly healthy individuals according to laboratory guidelines,[18] which recommends inclusion of > 120 people per group. However, this is not feasible for all patient categories. Therefore, laboratories sometimes try to calculate their cut-off values based on method comparison studies. In less well-standardised tests this is more challenging. Moreover, here the clinical application and discriminating capability of the test to differentiate between different causes of hyperthyroidism is more relevant. Therefore, ROC curve analysis was applied as was performed in previous studies.

Our analysis revealed a cut-off value of 4.5 IU/l, with 96% sensitivity and 91% specificity. The sensitivity and specificity are comparable to other assays,[19] whereas the cut-off value of 3.3 IU/l proposed by the manufacturer had 79% specificity, much lower compared to other assays.[19] Of note, the cut-off value of 3.3 IU/l had 100% sensitivity. However, although extremely rare, some Graves' patients have negative TRAb concentrations, sometimes even below 2.9 IU/l.[20] Therefore, aiming for a cut-off level with 100% sensitivity is not favourable. Extending the grey zone is agreement with findings of another study by Villalta et al. that identified a cut-off value of 3.8 IU/l.[21]

As mentioned above, the manufacturer determined the threshold in an internal study (n = 400, including patients with Graves' disease, Hashimoto's thyroiditis, non-autoimmune thyroid diseases, and a variety of non-thyroid diseases).[14] The group of Graves' patients consisted of a mixture of patients that were newly diagnosed and patients under treatment. Of note, in our study population, if we combine treated and newly diagnosed Graves' patients, the cut off value was 3.4 IU/l, comparable to the manufacturer's (figure 4). As shown in figure 2 and consistent with other studies,[15] patients under treatment have lower antibody concentrations, suggesting a possible explanation for the observed difference in cut-off values found by Villalta et al. and us, compared to the manufacturer's cut-off. One advantage of implementing a higher cut-off value is that the variance around the threshold concentration will be lower as the CV decreases with increasing antibody concentrations. This is important as measurements with a concentration near the threshold value should be as precise as possible to limit the number of false positives and false negatives. In this study, we have shown that the analysed assay (Thermofisher Scientific, Phadia) has a relatively high CV, with CV > 20% at a concentration below the threshold. Using the reported variation by the manufacturer, 15% at cut-off value, this means that cut-off value of 3.3 IU/l can range from ~ 2.3-4.3 IU/l (95% confidence interval). This is also important as some patients with thyroiditis had positive TRAb. However, these patients showed TRAb concentrations within in the 95% confidence interval of the cut-off value. Therefore, the positive results of these patients with thyroiditis are uncertain because of the measurement variation.

The complexity of the diagnosis of Graves' disease includes two aspects. First, the absence of consistent recommendations on how to diagnose Graves' disease. Second, clinical symptoms and imaging tools cannot always solely be used to diagnose Graves' disease and there is no single feature to discriminate Graves' disease from other causes of hyperthyroidism. For example, diffuse uptake on a scintigraphy fits the diagnosis of Graves, whereas this feature can also resemble recovering thyroiditis. Therefore, it is the clinician's overall judgement - which can include clinical follow up - that determines the diagnosis. The difficulty to discriminate Graves' disease from other causes of hyperthyroidism underscores the importance of TRAb measurements. Along those lines, this also emphasises the importance of using correct cut-off values for TRAb assays, as most diagnoses in clinical practice are dependent on the outcome of this assay.

One limitation of this study is that, as arguments for diagnosis may include positive TRAb, selection bias cannot be excluded. However, 6/7 patients with antibody

concentrations below 6.0 IU/l showed radiography results resembling Graves' disease. Another question that arises is: which assay should be used to measure TRAb? Assays that measure TRAb are mainly divided into three categories: 1) competitive immunoassays are assays in which the autoantibodies in the patient's serum compete with either recombinant TSH or a monoclonal antibody against the TSH receptor. Within this assay group, different generations exist and all cannot discriminate between stimulating and blocking antibodies. Of note, this leads to one limitation of this assay category: if another cause of hyperthyroidism is present, there is a possibility of misdiagnosing Graves' disease in a patient with blocking antibodies. 2) Bioassays in which cyclic adenosine monophosphate production is detected upon incubation with a patient's serum. These assays specifically measure stimulating antibodies, not blocking antibodies. 3) Recently developed bridge immunoassays are assays designed to specifically measure stimulating antibodies. As hyperthyroidism in Graves' disease is caused by stimulating antibodies one could argue that the novel bridge assays are best to use.[22] However, bridge assays cannot be used for all patient groups. For example, in pregnant women, presence of TRAb predicts the chance of thyroid dysfunction in the foetus.[4] In this specific setting, blocking antibodies are also relevant since they can cause hypothyroidism of the foetus. Another complicating factor is that it is possible that patients switch between stimulating and blocking antibodies.[23] Therefore, it is not recommended to measure only stimulating antibodies in patients with known stimulating antibodies.

When using guidelines, it is important to realise that thresholds mentioned in these guidelines cannot be applied uniformly by all laboratories. For example, the Dutch guideline for obstetrics and gynaecology recommends monitoring of the foetus when TRAb concentrations in pregnancy are above 5-10 IU/l.[10] This threshold is based on a method from the 1990s that is no longer widely used in the Netherlands. As explained above, different assays are not comparable. The international guideline recommends monitoring when the TRAb concentration is more than 2-3-fold above the threshold.[11] This type of recommendation is more suitable; however, any new assay requires determination of relevant cut-off values for different patient groups. Reference values cannot be calculated from another method by a simple formula due to lack of standardisation among assays.

In conclusion, the cut-off value of a TRAb assay should be based solely on newly diagnosed patients, excluding patients under treatment. For this immunoassay, the grey zone proposed by the manufacturer should probably be extended in untreated Graves' patients. Lastly, clinical context and expertise of the clinician remain crucial in the workup of patients with possible Graves' disease.

ACKNOWLEDGEMENTS

The authors thank G. Verloop and K. v.d. Laan for their contribution to the TRAb measurements.

DISCLOSURES

All authors declare no conflicts of interest. No funding or financial support was received.

REFERENCES

1. Smith TJ, Hegedus L. Graves' Disease. N Engl J Med. 20160;375:1552-65.
2. Tozzoli R, Bizzaro N. Harmonization in autoimmune thyroid disease diagnostics. Clin Chem Lab Med. 2018;56:1778-82.
3. Diana T, Olivo PD, Kahaly GJ. Thyrotropin Receptor Blocking Antibodies. Horm Metab Res. 2018; 50:853-62.
4. Barbesino G, Tomer Y. Clinical review: Clinical utility of TSH receptor antibodies. J Clin Endocrinol Metab. 2013;98:2247-55.
5. Frohlich E, Wahl R. Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and Extra-Thyroidal Diseases. Front Immunol. 2017;8:521.
6. Saravanan P, Dayan CM. Thyroid autoantibodies. Endocrinol Metab Clin North Am. 2001; 30:315-37, viii.
7. Zophel K, Roggenbuck D, Schott M. Clinical review about TRAb assay's history. Autoimmun Rev. 2010;9:695-700.
8. Ross DS, Burch HB, Cooper DS, et al. 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid. 2016; 26:1343-421.
9. NIV. Richtlijn Schildklierfunctiestoornissen. 2012 [Assessed on 27th of July 2019]. Available from: https://www.internisten.nl/sites/internisten.nl/files/uploads/le/yt/leytn7i-HoCwudu3Yz40Og/Conceptrichtlijn_2012_Schildklierfunctiestoornissen-2012.pdf
10. NVOG. Richtlijn schildklier en zwangerschap. 2010. [Assessed on 27th of July 2019] Available from: https://www.nvog.nl/wp-content/uploads/2017/12/Schildklier-en-zwangerschap-2.0-04-06-2010.pdf
11. De Groot L, Abalovich M, Alexander EK, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97:2543-565.
12. Massart C, d'Herbomez M. Thyroid-stimulating hormone receptor antibody assays: recommendation for correct interpretation of results in Graves disease. Clin Chem. 2013;59:855.
13. Pedersen IB, Handberg A, Knudsen N, Heickendorff L, Laurberg P. Assays for thyroid-stimulating hormone receptor antibodies employing different ligands and ligand partners may have similar sensitivity and specificity but are not interchangeable. Thyroid. 2010;20:127-33.
14. Internal study by ThermoFisher.
15. Laurberg P, Wallin G, Tallstedt L, Abraham-Nordling M, Lundell G, Torring O. TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. Eur J Endocrinol. 2008;158:69-75.
16. Hesarghatta Shyamasunder A, Abraham P. Measuring TSH receptor antibody to influence treatment choices in Graves' disease. Clin Endocrinol (Oxf). 2017;86:652-7.

17. Pedersen IB, Knudsen N, Perrild H, Ovesen L, Laurberg P. TSH-receptor antibody measurement for differentiation of hyperthyroidism into Graves' disease and multinodular toxic goitre: a comparison of two competitive binding assays. Clin Endocrinol (Oxf). 2001;55:381-90.

18. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition. CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.

19. Tozzoli R, Bagnasco M, Giavarina D, Bizzaro N. TSH receptor autoantibody immunoassay in patients with Graves' disease: improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis. Autoimmun Rev. 2012;12:107-13.

20. Paunkovic J, Paunkovic N. Does autoantibody-negative Graves' disease exist? A second evaluation of the clinical diagnosis. Horm Metab Res. 2006;38:53-6.

21. Villalta D, D'Aurizio F, Da Re M, Ricci D, Latrofa F, Tozzoli R. Diagnostic accuracy of a new fluoroenzyme immunoassay for the detection of TSH receptor autoantibodies in Graves' disease. Auto Immun Highlights. 2018;9:3.

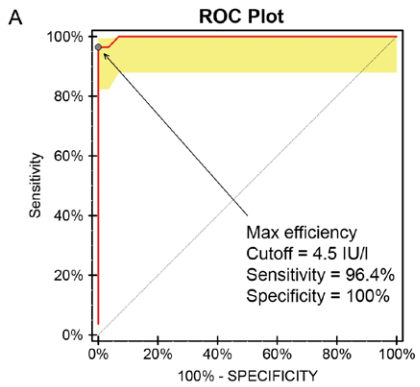
22. Frank CU, Braeth S, Dietrich JW, Wanjura D, Loos U. Bridge Technology with TSH Receptor Chimera for Sensitive Direct Detection of TSH Receptor Antibodies Causing Graves' Disease: Analytical and Clinical Evaluation. Horm Metab Res. 2015;47:880-8.

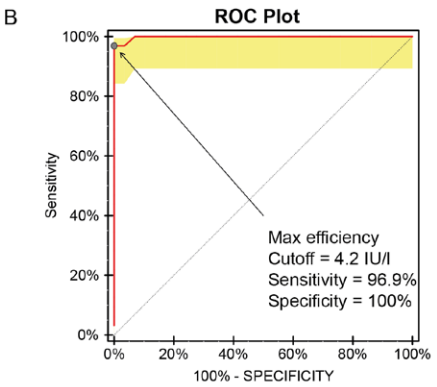
23. McLachlan SM, Rapoport B. Thyrotropin-blocking autoantibodies and thyroid-stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. Thyroid. 2013;23:14-24.

Supplementary table 1. *Coefficient of variation (CV) for three different samples*

Sample	Concentration (mean)	Concentration (min-max)	CV (within run)	CV (between run)	CV (total)
A	2.2 IU/l	1.6-3.6 IU/l	16.4%	19.1%	26.4%
B	9.6 IU/l	7.9-12 IU/l	14.2%	10.3%	16.7%
C	22 IU/l	17-28 IU/l	12.3%	9.0%	15.2%

Supplementary figure 1. *ROC curve analysis of different patient groups where patients with MNG and positive TRAb were either excluded from the analysis or included in the Graves' disease group.*
A. ROC curve analysis of 57 patients, where patients with MNG and positive TRAb (n = 4) were excluded. The study population consisted of 28 patients with newly diagnosed Graves' disease, 11 patients with multinodular goitre and/or toxic adenoma, and 18 patients with thyroiditis. B. ROC curve analysis of 61 patients, where patients with MNG and positive TRAb (n = 4) were included in the positive cases. The study population consisted of 32 patients with newly diagnosed Graves' disease, 11 patients with multinodular goitre and/or toxic adenoma, and 18 patients with thyroiditis.





N=57	Negative cases	Positive cases
Samples	11 MNG/toxic adenoma 18 Thyroiditis	28 Graves' disease
Range	1.5 to 3.7 IU/l	3.4 to 52 IU/l

N=61	Negative cases	Positive cases
Samples	11 MNG/toxic adenoma 18 Thyroiditis	28 Graves' disease 4 MNG
Range	1.5 to 3.7 IU/l	3.4 to 52 IU/l

MNG = multinodular goitre; N = number of patients; ROC = receiver operating characteristic

ORIGINAL ARTICLE

Hyperactive thyroid nodules treated by radiofrequency ablation: a Dutch single-centre experience

H. de Boer[1]*, W. Bom[1], P. Veendrick[2], E. Bom[2], M. van Borren[3], F. Joosten[2]

Departments of [1]Internal Medicine, [2]Radiology, [3]Clinical Chemistry, Rijnstate Hospital, Arnhem, the Netherlands. *Corresponding author: hdeboer@rijnstate.nl

KEYWORDS

Hyperthyroidism, radiofrequency ablation, toxic thyroid nodules

ABSTRACT

Background: Hyperactive thyroid nodules (HTN) are usually treated with radioactive iodine (RAI). However, as RAI is associated with a 30-60% long-term risk of permanent hypothyroidism, radiofrequency ablation (RFA) may be a good alternative. Primary aim of this study was to assess the percentage of patients achieving euthyroidism after RFA.

Patients and Methods: Patients with a symptomatic HTN were treated by ultrasound-guided RFA, using the trans-isthmic approach and moving-shot technique, in an outpatient setting under local anaesthesia.

Results: Twenty-one patients were included, ranging in age from 37-75 years. Follow-up was at least one year. All patients had a suppressed serum thyroid-stimulating hormone (TSH), with free thyroxine (FT4) and free triiodothyronine (FT3) concentrations mildly elevated in 33% and 43% of cases, respectively. RFA was not associated with clinically meaningful adverse effects. TSH normalisation was achieved in 11/21 patients (52%) after first RFA. A partial response, defined as a normalisation of FT4 and FT3, but incomplete improvement of TSH, was observed in 6/21 patients (29%). Three patients had no response (14%), and one patient developed mild, asymptomatic subclinical hypothyroidism. Five patients underwent a second RFA and this led to TSH normalisation in four, thereby raising the rate of complete remission to 71%. Recurrence of TSH suppression did not occur during the study period.

Conclusion: These data suggest that RFA is a safe and promising treatment for symptomatic hyperactive thyroid nodules, with a low risk of permanent hypothyroidism. Long-term studies are needed to identify the recurrence risk of hyperthyroidism.

INTRODUCTION

Hyperactive thyroid nodules (HTNs) are autonomous functioning thyroid adenomas (AFTN) that produce excessive amounts of thyroid hormone. About 5-10% of all palpable solitary nodules are hyperactive.[1] HTNs develop slowly over a period of years, and gradually increase their contribution to overall thyroid hormone secretion as they grow. Ultimately, this leads to (subclinical) hyperthyroidism and mostly mild thyrotoxicosis for nodules < 2.5 cm, while overt thyrotoxicosis occurs in 80% of HTNs > 5 cm.[1-3] The rate of progression to thyrotoxicosis in previously euthyroid patients with HTN is about 4% per year. Atrial fibrillation and progressive osteoporosis are the main complications. The risk of malignancy in an HTN is extremely low.[1-3]

Until recently, radioactive iodine (RAI), surgery, and anti-thyroid drugs (ATD) have been the primary treatment options for HTNs. Treatment with ATD is not recommended as first choice because recurrence of hyperthyroidism is sure to occur after discontinuation of treatment. ATDs are mainly used to temporarily stabilise patients before they receive a more permanent therapy, or in patients with limited life expectancy, and in cases where other treatment options are not feasible or contraindicated. Surgery, i.e., unilateral lobectomy, is rarely performed despite the advantage of rapid and permanent biochemical control, with euthyroidism within several days, and recurrence or persistence of hyperthyroidism

in fewer than 1% of patients.[4] Information about the risk of hypothyroidism after lobectomy for HTN is very limited. The weighted mean incidence of post-operative hypothyroidism after lobectomy for benign thyroid disease in general is 22% (95% confidence interval 18-27%).[5] Whether similar figures are to be expected after lobectomy for HTN is currently not known. Administration of RAI is the treatment of first choice in most countries, mainly because of its non-invasive nature and high cure rates. Euthyroidism is commonly achieved within 3-6 months in 90-95% of patients and is accompanied by a mean nodule volume reduction of 30-45%.[6] The most important limitation of radioactive iodine (^{131}I) treatment is its high risk of post-irradiation hypothyroidism. Although early studies using a fixed activity of 740 MBq have reported a prevalence of 5-10%, more recent long-term studies have reported a 10-year risk of hypothyroidism of 30-40% despite the use of ^{131}I activities of 500-550 MBq, and a 20-year risk of 60%.[7-12] Cure rates and risk of post-irradiation hypothyroidism after ^{131}I treatment vary considerably among studies, and depend on the size of the nodule, the activity applied, the use of fixed activity versus calculated activity based on dose per gram thyroid tissue, the degree of extra-nodular thyroid tissue suppression, ATD pre-treatment, and the presence of thyroid antibodies.[9-11]

More recent techniques for the treatment of HTNs include ultrasound (US)-guided percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA). In very experienced hands, euthyroidism has been achieved in 70-90% of patients, with a less than 1% risk of posttreatment hypothyroidism during a median follow-up of 2.5-5 years,[13,14] However, today most centres have abandoned the use of PEI because of its patient burden caused by the multiple treatment sessions (5 to 15 max) required to achieve euthyroidism, concern about the risk of ethanol diffusion outside the thyroid capsule causing local nerve or vascular damage, and the relatively high recurrence rate of hyperthyroidism after long-term follow- up.[4,12]

Treatment of HTNs by RFA has been introduced by two different groups at about the same time in 2008.[15,16] Deandrea et al. used a fixed electrode position in the node's centre, whereas Baek et al. developed the so-called moving shot technique where the electrode is moved under US guidance to produce multiple small, heat-induced ablation zones throughout the thyroid node.[17] The moving shot technique has become the technique of first choice because of its higher efficacy.[16] The extensive 10-year experience with moving shot RFA for non-functioning benign thyroid nodules has shown that it is capable of reducing nodal volume by 50-80%, at a very low complication rate.[18,19] Global RFA experience with the aim to induce euthyroidism in patients with HTN is still limited. As of July 2019, a total of only 167 cases have been reported by centres in South Korea, China, Italy, and Austria. These studies have shown a risk of hypothyroidism of less than 3%.[20-25] In the present study, we describe our experience of the first 21 patients treated in our centre. Primary aim was to establish the percentage of patients achieving a normalisation of serum free thyroxine (FT4), free triiodothyronine (FT3), and thyroid stimulating hormone (TSH) during a follow-up of one year. Nodal volume reduction and adverse effects were secondary outcome parameters.

PATIENTS AND METHODS

All consecutive patients presenting with symptomatic HTNs between May 2015 and May 2018 were included in this study. Inclusion criteria were: 1) symptoms suggestive of hyperthyroidism; 2) documented biochemical hyperthyroidism, defined as a suppression of serum TSH below the lower limit of normal (LLN: 0.3 mU/l), with FT4 or FT3 levels within or above the population normal range; 3) absence of TSH-receptor antibodies (TRab); 4) presence of a single hot nodule on ^{123}I scintigraphy, corresponding with a well-demarcated nodule by US with a diameter of 20-50 mm; and 5) a post-RFA follow-up of at least one year. All patients meeting the inclusion criteria received verbal and written information about reported cure rates and adverse effects of RAI therapy and RFA and were free to choose either procedure. Without exception, all patients preferred treatment by RFA, mainly because they wished to avoid hypothyroidism. The study was approved by the local ethical committee (study number 2018-1335), and all patients gave their informed consent.

Laboratory tests

Serum TSH, FT4, and FT3 concentrations were measured on a Modular E170 (Roche Diagnostics, Almere, The Netherlands), and TSH receptor antibodies were measured on an Immulite 2000 (Siemens Healthcare, Den Haag, The Netherlands). Reference values were: TSH 0.30-4.2 mU/l; FT4 12-22 pmol/l; FT3 3.1-6.8 pmol/l; TRab < 1.0 U/l. The TSH detection limit was 0.005 mU/l.

Ultrasound

Thyroid imaging by ultrasound was performed by one of three dedicated thyroid radiologists, using a 12.5 MHz linear probe (Epic 5G, Philips Medical systems, Best, The Netherlands). Nodule dimensions and volume were assessed by measurement of three orthogonal nodule diameters (a, b, and c). The nodule volume was calculated by the equation: $V = {}^{1}/_{6}\ (\pi abc)$, where a represents

the maximum diameter, and b and c, the other two perpendicular diameters. Nodule composition was assessed subjectively, and classified as solid if the solid tissue component was > 75%, and as cystic if the cystic component was > 75%. All other nodules were classified as mixed type.

^{123}I scintigraphy

Thyroid imaging was performed with ^{123}I in a dose of 15 MBq and assessment of 24-hour uptake with a high-energy, high-resolution collimator (Brightview XCT v2.5, Philips Medical Systems, Best, The Netherlands). Two types of images were observed: images with a single hotspot without any uptake in surrounding thyroid tissue (complete suppression), and images with a single hot spot with some uptake in the surrounding thyroid tissue (partial suppression).

RFA procedure

Platelet inhibitors were discontinued seven days before RFA, vitamin K antagonists three to five days, and DOACs 48 hours prior to treatment. RFA was performed in an outpatient day care setting, under local anaesthesia. Anxious patients received oxazepam 10 mg orally upon request, one hour before the procedure. Local anaesthesia was achieved by a US-guided lidocaine 2% injection in the skin and around the thyroid capsule. Mixed and cystic nodules were first treated by aspiration of all cyst fluid to obtain a solid nodule amenable to RFA. RFA was performed by one of three dedicated radiologists, with a Viva RF generator (STARmed Seoul, South Korea) and an 18-gauge internally-cooled electrode with a 10 mm active tip (Star RF Electrode, STARmed, Seoul, South Korea), using the trans-isthmic approach and the so-called moving shot technique, as described previously.[17] The applied power ranged from 30 to 50 Watt. Voice testing and assessment of pain sensation was performed frequently during the RFA procedure to avoid any damage due to overheating of surrounding tissues. After completion of RFA, all patients remained under observation for two hours in the day care ward. Oral paracetamol 1000 mg four times daily was prescribed for one day.

Follow-up

Follow-up after RFA included measurements of FT4, FT3, and TSH at 1, 4, 13, 26, and 52 weeks, and measurement of nodule volume by ultrasound at 13, 26, and 52 weeks. Repeat ^{123}I scintigraphy was performed at 52 weeks.

Statistics

Results are shown as mean values ± standard deviation or as median values with range. The effects of treatment over time were analysed by the Kruskal-Wallis and the Wilcoxon rank-sum tests. P values < 0.05 were considered as statistically significant.

RESULTS

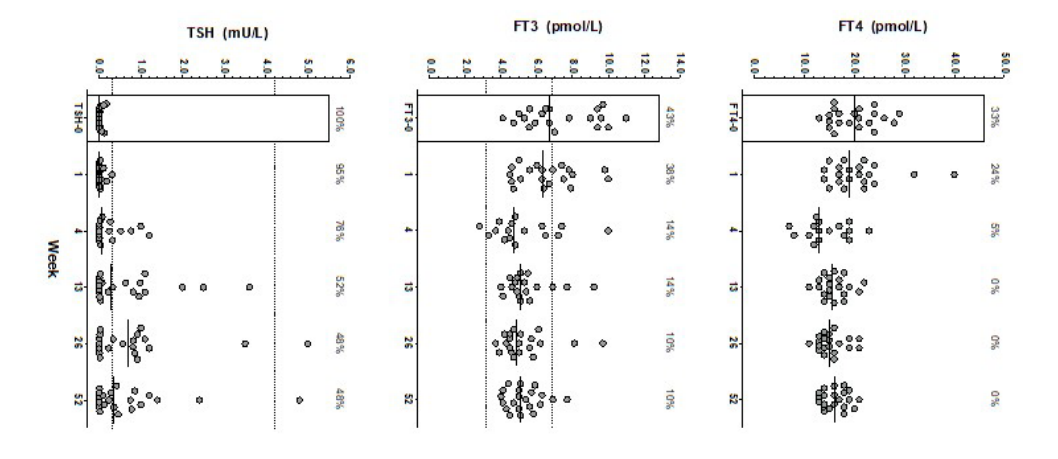
Table 1. *Baseline characteristics of 21 patients with a symptomatic solitary toxic thyroid nodule included for treatment with radiofrequency ablation*

	Mean ± SD or median (Range)
Patients (N)	21
Age (yrs)	58.2 ± 9.8
Height (cm)	165 ± 9.8
Weight (kg)	67.0 ± 10.9
Symptoms	21 (100%)
- Nervousness	12 (57%)
- Palpitations	12 (57%)
- Fatigue	6 (29%)
- Heat intolerance	6 (29%)
- Weight loss	3 (14%)
- Tremor	2 (10%)
Osteopenia/Osteoporosis	7 (33%)
Atrial fibrillation	4 (19%)
Thyroid nodule volume (ml)	9.8 (1.0-60)
Maximal nodule diameter (mm)	37 (11-56)
TSH (mU/l)	0.0 ± 0.1
FT4 (pmol/l)	18.9 ± 4.5
FT3 (pmol/l)	6.8 ± 2.0

FT3 = free triiodothyronine; FT4 = free thyroxine; TSH = thyroid stimulating hormone

A total of 21 patients were included. Their baseline characteristics are shown in table 1. All patients had at least one symptom suggestive of hyperthyroidism. Nervousness and palpitations were most common, followed by fatigue, heat intolerance, weight loss, and tremor. One-third had a diagnosis of osteopenia or osteoporosis, and one-fifth had atrial fibrillation for which they received a beta blocker and oral anticoagulation by DOAC. Ten patients had incomplete suppression of extra-nodular thyroid tissue. The majority of patients were treatment naïve; only two patients had received pre-treatment with thiamazole 5 mg daily, but this

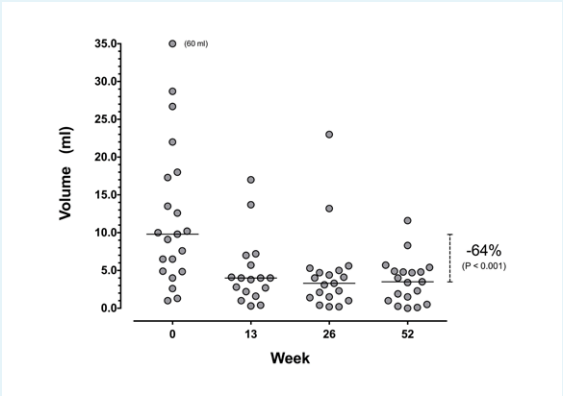
Figure 1. *Changes in thyroid hormone and TSH levels after single RFA of hyperactive thyroid nodules, during a one-year follow-up. The upper and lower limits of normal are shown as interrupted lines. The percentages of patients with either elevated FT4 and FT3 levels or suppressed TSH levels during follow-up are shown in the top of each figure.*



was discontinued one week before RFA. Follow-up after RFA ranged from 1.3-4.1 years, with a median of 2.5 years.

At baseline, all patients had serum TSH levels below the LLN, with TSH levels below assay detection limit in 8/21 (38%) patients. FT4 and FT3 levels were above the upper limit of normal in 33% and 43% of patients, respectively (figure 1). Maximal nodule diameters at baseline ranged from 11-56 mm (median 37 mm), with nodule volumes ranging from 1.0-60 ml and a median of 9.8 ml (figure 2). Five patients had central cystic degeneration, and 4-12 ml cyst fluid (median 5.0 ml) was aspirated to obtain a solid nodule amenable to RFA treatment. RFA was performed with a power of 30-55 Watt (median 45 Watt), applying a median of 4.4 kcal (range 1.5-10 kcal). Procedure duration ranged from 3-16 minutes with a median of 10 min. Major volume reduction was observed after three months, with stabilisation after six months. The median volume reduction at 12 months was 64% ($p < 0.0001$, figure 2). This decreased to 61% after correction for fluid aspiration in the five patients with central cystic degeneration. The decline in volume was directly proportional to the amount of energy applied ($r = 0.74$, $p < 0.005$). Two patients had worsening of hyperthyroidism due to RFA-induced thyroiditis, with FT4 levels rising from 24 to 40 pmol/l and from 21 to 32 pmol/l, respectively, in the first week after treatment (figure 1). This was followed by mild but persistent, antibody-negative hypothyroidism in the first patient, which required levothyroxine 50 mg daily to achieve normal TSH levels. Spontaneous recovery occurred in the second patient within four weeks. Another two patients developed transient post-thyroiditis hypothyroidism with FT4 nadir levels of 8 and 11 pmol/l, but with full recovery within four weeks. The main reduction of thyroid hormone levels was achieved after three months, with only minimal changes thereafter. At 12 months, FT4 and FT3 levels were within the normal range in 100% and 90% of patients, respectively, and TSH had increased to levels within the normal range in 11 patients (52%) without medication, and in one patient under treatment with levothyroxine 50 mg daily. The other nine patients had persistent TSH suppression. Two had become asymptomatic and did not wish additional treatment. Seven had mild but persistent symptoms of hyperthyroidism. One chose to be treated with RAI and became euthyroid after a dose of 196 MBq,

Figure 2. *Volume changes of hyperactive thyroid nodules after single RFA*



and one patient preferred thiamazole 5 mg monotherapy. A second RFA was performed in five patients, and this normalised TSH levels in four out of five. This raised the post-RFA remission rate to 71%. Recurrence of hyperthyroidism was not observed during follow-up.

Repeat ^{123}I scintigraphy was performed in 15 patients one year after RFA. At 12 months, 24-hour ^{123}I uptake had decreased from 27 to 19%. Normalisation of uptake distribution in both thyroid lobes occurred in five patients. Hot nodule uptake remained visible in 10 patients, with unchanged suppression of the contralateral lobe in five patients and improved contralateral uptake in the remaining five patients.

Adverse effects

During the procedure, transient mild pain at the site of treatment was common, sometimes radiating to the lower jaw or ear. Pain rapidly disappeared after brief interruption of the procedure, lowering of wattage, or repositioning of the electrode. Other adverse effects were not observed. Any post-procedural pain was well controlled with paracetamol 1000 mg four times a day, and most patients stopped this medication after one day. There were no long-term adverse effects.

DISCUSSION

This study shows that RFA treatment of HTNs is a feasible option. It induced permanent euthyroidism in 52% of patients after the first RFA and this increased to 71% after a second RFA. Only one patient developed mild, but permanent subclinical hypothyroidism with a peak serum TSH of 8.0 mU/l. Clinically-meaningful adverse effects other than transient procedural pain were not observed.

Our results are in agreement with previous studies also using the trans-isthmic approach and moving shot technique. These data are summarised in table 2, and include 188 patients with a follow-up of 12-24 months.[15,20-25] Although overall global experience is still limited, the data published so far indicate that euthyroidism can be achieved in about 75% of patients (range 50-91%) and at a very low risk of hypothyroidism of only 2.7%. About 30% percent of patients required more than one treatment to achieve euthyroidism and about 10% had more than two treatments. The main differences among these moving shot studies are related to the percentage of patients with overt thyrotoxicosis, presence or absence of ATD pre-treatment, mean nodule volumes at inclusion, and varying operator experience.

The efficacy of RFA appears to be primarily determined by RFA technique and nodule size. Initially, several studies were performed with a fixed needle position in the node's centre.[16,26-28] This technique is no longer used because of its limited efficacy with a median TSH normalisation rate of only 39% (range 21-54%), which is much lower than achieved with the moving shot technique.[26] The impact of nodule size was evaluated in a single centre study of 29 patients with HTN. A single RFA treatment induced euthyroidism in 86% of patients with small nodules (mean volume of 5 ml, range 3-8 ml), but only induced euthyroidism in 45% of patients with large HTNs (mean volume 18 ml, range 12-29 ml).[23] Whether a second RFA procedure can improve the cure rate of large nodules was not reported.

The ultimate goal of treatment is to achieve sustained normalisation of thyroid hormone levels and TSH without medication and without the need of follow-up. This implies that the definition of complete cure should not be limited to achievement of biochemical euthyroidism but should also include normalisation of ^{123}I uptake in both thyroid lobes. In our study, only 30% of patients met these criteria. Others reported higher complete cure rates of up to 87%, and this may be related to a smaller nodule size as well as a greater operator experience. The biochemically euthyroid patients with persistent HTN remnant uptake may be at risk to develop a recurrence of hyperthyroidism. This subset of patients will require yearly follow-up, and possibly a second RFA in due time. Persistent HTN uptake with incomplete recovery of the contralateral lobe uptake has been observed in 45% of patients (range 13-86%) that have been treated worldwide (table 2). Although recurrence of hyperthyroidism has not been reported so far, it is likely to occur sometime. Structured long-term follow-up will be important to obtain the data required for evidence-based decisions about the optimal mode of treatment in specific cases. Ablation can be expected to be incomplete in nodules positioned in the vicinity of vital structures, such as the recurrence nerve and carotid artery or jugular vein, because these so-called danger areas need to be avoided. To reduce the risk of incomplete ablation and recurrence of HTN, it may be useful to include US flow measurements as standard procedure and to proceed with RFA until all nodule flow has disappeared.

The major advantage of RFA for HTNs is its very low risk of post-treatment hypothyroidism and the lack of radiation exposure. Out of 188 cases, only five (2.7%) developed hypothyroidism, and in all but one case, it was mild with TSH levels less than 10 mU/l (range 4.9-8.0 mU/l). De novo anti-TPO antibodies were detected in two of these subjects. So far, follow-up after RFA is two years or less in most cases. We therefore do not know whether the incidence of post-RFA hypothyroidism will increase on the long-term. However, this is considered to be unlikely because RFA is a local treatment, sparing extra-nodal thyroid tissue.

Table 2. *Summary of reported RFA treatments for HTN in an outpatient setting, under local anaesthesia, with an internally cooled electrode, introduced by trans-isthmic approach and performed with the moving shot technique*

Reference	Patients (N)	Follow-up (Months)	Median volume (ml)	Number of sessions N	Biochemical remission (N / %)	Volume reduction (%)	Persistence of extra-nodular suppression	Hypo-T (N)
Baek 2008	1	19	5.1	1	1 (100%)	97%	0	0
Baek 2009	9	6	15.0	1-4	5 (56%)	71%	56%	1*
Sung 2015	44	6-56	18.5	1-6	36 (82%)	82%	20%	0
Bernardi 2017	30	12	17.1	1	15 (50%)	75%	NR	0
Cesareo[A] 2018	15	24	5.2	1	13 (86%)	84%	13%	0
Cesareo[B] 2018	14	24	18.3	1	6 (45%	68%	86%	0
Dobnig 2018	32	12	14.3	1	27 (84%)	86%	NR	1
Cervelli 2019	22	12	14.3	1	20 (91%)	76%	NR	2
Present study	21	12	9.8	1-2	15 (71%)	64%	67%	1
All	188		14.8		118 (74%)	77%	45%	5 (2.7%)

[A] nodules < 12 ml; [B] nodules > 12 ml; * pre-existent anti-TPO antibodies; Hypo-T = hypothyroidism; NR = not reported

As discussed previously, normalisation of thyroid hormone levels can also be achieved with surgery or RAI treatment, however, this is associated with a relatively high risk of hypothyroidism, of up to 30% after lobectomy and to about 30-60% after RAI treatment. This implies that patients treated by surgery or RAI will require laboratory follow-up for several years, and if they develop hypothyroidism, levothyroxine treatment and biochemical monitoring will be needed for life. Prolonged follow-up is unlikely to be necessary after successful RFA, however, long-term data will be needed to prove that point. With these considerations in mind, a reappraisal of the currently available treatment modalities for HTN seems to be warranted.

In conclusion, results published on RFA so far, indicate that RFA may be considered as a candidate treatment of first choice for HTN. Euthyroidism can be expected in about 75% of patients, with a less than 3% risk of hypothyroidism. Currently available data suggest that surgery might be preferable in large HTNs and that RAI might be used as treatment in patients where surgery or RFA is contraindicated, or RFA fails to restore euthyroidism. However, the optimal indications for each of these treatments remain to be determined in future studies with long-term head-to-head comparison of RFA and RAI.

DISCLOSURES

All authors declare no conflicts of interest. No funding or financial support was received.

REFERENCES

1. Corvilain B. The natural history of thyroid autonomy and hot nodules. Ann Endocrinol. 2003;64:17-22.
2. Hamburger JL. The autonomously functioning thyroid nodule; Goetsch's disease. Endocr Rev 1987;8:439-47.
3. Sandrock D, Olbricht T, Emrich D, Benker G, Reinwein D. Long-term follow-up in patients with autonomous thyroid adenoma. Acta Endocrinol. 1993;128:51-5.

4. Ross DS, Burch HB, Cooper DS, et al. American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid. 2016;1363-9.
5. Verloop H, Louwerens M, Schoones JW, Kievit J, Smit JWA, Dekkers OM. Risk of hypothyroidism following hemithyroidectomy: Systematic review and meta-analysis of prognostic studies. J Clin Endocrinol Metab. 2012;97:2243-55.
6. Bonnema SJ, Hegedus L. Radioiodine therapy in benign thyroid disease: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33:920-80.
7. Huysmans DA, Corsten FH, Kloppenborg PW. Long-term follow-up in toxic solitary autonomous thyroid nodules treated with radioactive iodine. J Nucl Med. 1991;32:27-30.
8. Nygaard B, Hegedus L, Nielsen GK, Ulriksen P, Hansen JM. Long-term effect of radioactive iodine on thyroid function and size in patients with solitary autonomously functioning toxic thyroid nodules. Clin Endocrinol. 1999;50:197-202.
9. Ahmad AM, Ahmad M, Young ET. Objective estimates of the probability of developing hypothyroidism following radioactive iodine treatment of thyrotoxicosis. Eur J Endocr. 2002;146:767-75.
10. Ceccarelli C, Bencivelli W, Vitti P, Grasso L, Pinchera A. Outcome of radioiodine-131 therapy in hyperfunctioning nodules: a 20 years' retrospective study. Clin Endocrinol. 2005;62:331-5.
11. Filesi M, Travascio L, Montesano T, et al. The relationship between 24h/4h radioiodine-131 uptake ratio and outcome after radioiodine therapy in 1402 patients with solitary autonomously functioning thyroid nodules. Ann Nucl Med. 2009;23:229-34.
12. Yano Y, Sugino K, Akaishi J, et al. Treatment of autonomously functioning thyroid nodules at a single institution: radioiodine therapy, surgery, and ethanol injection therapy. Ann Nucl Med. 2011;25:749-54.
13. Monzani F, Caraccio N, Goletti O, et al. Five-year follow-up of percutaneous ethanol injection for the treatment of hyperfunctioning thyroid nodules: a study of 117 patients. Clin Endocrinol. 1997;46-9.
14. Trantino L, Francica G, Sordelli I, et al. Percutaneous ethanol injection of hyperfunctioning thyroid nodules: long-term follow-up in 125 patients. Am J Radiol. 2008;190:800-8.
15. Baek JH, Jeong HJ, Kin YS. Radiofrequency ablation for an autonomously functioning thyroid nodule. Thyroid. 2008;18: 675-6.
16. Deandrea M, Limone P, Basso E, et al. US-guided percutaneous radiofrequency thermal ablation for the treatment of solid benign hyperfunctioning or compressive thyroid nodules. Ultrasound Med Biol. 2008;34:784-91.
17. Shin JH, Baek JH, Ha EJ, Lee JH. Radiofrequency ablation of thyroid nodules: Basic principles and clinical application. Int J Endocrinol. 2012;2012:1-7.
18. Baek JH, Lee JH, Sung JY, et al. Complications encountered in the treatment of benign thyroid nodule with UD-guided radiofrequency ablation. Radiol. 2021;262:335-42.
19. Cesareo R, Andrea P, Valerio P, et al. Radiofrequency ablation for the measurement of thyroid nodules: a critical appraisal of the literature. Clin Endocrinol. 2017;87:639-48.
20. Baek JH, Moon WJ, Kim YS, Lee JH, Lee D. Radiofrequency ablation for the treatment of autonomously functioning thyroid nodules. World J Surg. 2009;33:1971-7.
21. Sung JY, Baek JH, Jung SL, et al. Radiofrequency ablation for autonomously functioning thyroid nodules: a multicenter study. Thyroid. 2015;25:112-6.
22. Bernardi S, Stacul F, Michelli A, et al. Twelve month efficacy of a single radiofrequency ablation on autonomously functioning thyroid nodules. Endocrine. 2017;57:402-8.
23. Dobnig H, Amrein K. Monopolar radiofrequency ablation of thyroid nodules: a prospective Austrian single center study. Thyroid. 2018;28:472-80.
24. Cesareo R, Naciu AM, Iozzino M, et al. Nodule size as predictive factor of efficacy of radiofrequency ablation in treating autonomously functioning thyroid nodules. Int J Hyperthermia. 2018;34:617-23.
25. Cervelli R, Mazzeo S, Boni G, et al. Comparison between radioiodine therapy and single-session radiofrequency ablation of autonomously functioning thyroid nodules: a retrospective study. Clin Endocrinol. 2019;90:608-16.
26. Ceasareo R, Palermo A, Benvenuto D, et al. Efficacy of radiofrequency ablation in autonomous functioning thyroid nodules. A systematic review and meta-analysis. Rev Endocr Metab Disord. 2019;20:37-44.
27. Spiezia S, Garberoglio R, Milone F, et al. Thyroid nodules and related symptoms are stably controlled two years after radiofrequency thermal ablation. Thyroid. 2009;19:219-25.
28. Faggiano A, Ramundo V, Assanti AP, et al. Thyroid nodules treated with percutaneous radiofrequency thermal ablation: a comparative study. J Clin Endocrinol Metab. 2012;97:439-45.

ORIGINAL ARTICLE

A Dutch consensus statement on the diagnosis and treatment of ANCA-associated vasculitis

E. Dirikgil[1], S.W. Tas[2], A. Rutgers[3], P.M.J. Verhoeven[4], J.M. van Laar[5], E.C. Hagen[6], J. Tekstra[5], A.E. L. Hak[2], P. van Paassen[7], M.R. Kok[8], R. Goldschmeding[5], B. van Dam[9], C.E. Douma[10], H.H.F. Remmelts[6], J.F. Sanders[3], J.T. Jonker[1], T.J. Rabelink[1], J.G.M.C. Damoiseaux[7], H.J. Bernelot Moens[11], W. J. W. Bos[1], Y.K.O. Teng[1]* on behalf of the Arthritis Research & Collaboration Hub consortium

[1]Leiden University Medical Center, Leiden, the Netherlands; [2]Amsterdam University Medical Center, Amsterdam, the Netherlands; [3]University Medical Center Groningen, Groningen, the Netherlands; [4]the Dutch Vasculitis Foundation, Silvolde, the Netherlands; [5]University Medical Center Utrecht, Utrecht, the Netherlands; [6]Meander Medisch Centrum, Amersfoort, the Netherlands; [7]Maastricht University Medical Center, Maastricht, the Netherlands; [8]Maasstad Ziekenhuis, Rotterdam, the Netherlands; [9]Medical Center Alkmaar, Alkmaar, the Netherlands; [10]Spaarne Hospital, Haarlem, the Netherlands;[11]Ziekenhuis Groep Twente, Hengelo, the Netherlands. *Corresponding author: y.k.o.teng@lumc.nl

ABSTRACT

Introduction: Despite the availability of several guidelines on the diagnosis and treatment of antineutrophil cytoplasmic antibody-associated vasculitis (AAV), clinical routine practice will only improve when an implementation strategy is in place to support clinical decision making and adequate implementation of guidelines. We describe here an initiative to establish national and multidisciplinary consensus on broad aspects of the diagnosis and treatment of AAV relevant to daily clinical practice in the Netherlands.

Methods: A multidisciplinary working group of physicians in the Netherlands with expertise on AAV addressed the broad spectrum of diagnosis, terminology, and immunosuppressive and non-immunosuppressive treatment, including an algorithm for AAV patients. Based on recommendations from (inter)national guidelines, national consensus was established using a Delphi-based method during a conference in conjunction with a nationally distributed online consensus survey. Cut-off for consensus was 70% (dis)agreement.

Results: Ninety-eight professionals were involved in the Delphi procedure to assess consensus on 50 statements regarding diagnosis, treatment, and organisation of care for AAV patients. Consensus was achieved for 37/50 statements (74%) in different domains of diagnosis and treatment of AAV including consensus on the treatment algorithm for AAV.

Conclusion: We present a national, multidisciplinary consensus on a diagnostic strategy and treatment algorithm for AAV patients as part of the implementation of (inter)national guideline-derived recommendations in the Netherlands. Future studies will focus on evaluating local implementation of treatment protocols for AAV, and assessments of current and future clinical practice variation in the care for AAV patients in the Netherlands.

KEYWORDS

ANCA-associated vasculitis, pauci-immune glomerulonephritis, recommendations

INTRODUCTION

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a rare systemic autoimmune disease affecting small vessels and includes three different entities: granulomatosis with polyangiitis (Wegener) (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (eGPA).[1] Untreated AAV is associated with high morbidity and mortality[2] and therefore early diagnosis and treatment is essential to reduce fatal outcome and prevent chronic damage. Simultaneously, the wide variety of presenting symptoms

related to AAV, together with its low prevalence make timely diagnosis challenging to clinicians.

Many randomised diagnostic and therapeutic studies in the past half century have transformed AAV from a fatal disease to a chronic (relapsing) disease.[3] Survival rates below 20% in untreated GPA patients, before the introduction of corticosteroids and cyclophosphamide (CYC) in the 1960s,[2] have now improved to one-year survival rates in 81-95% of GPA patients, with 73-83% of patients surviving after 5 years and 55-75% after 10 years.[4] Improved survival coincides with an increased risk of side-effects from intensive immunosuppression in the long-term, such as infections and malignancies. Therefore, current studies aim to improve and define the optimal balance between over- and under-immunosuppression during remission-induction and maintenance therapy.

AAV patients encompass a heterogeneous group that requires an individualised and often multidisciplinary approach to treatment. The clinical diagnosis, the severity of the disease, and patient characteristics are important for tailoring an optimal treatment strategy for each AAV patient.[5-8] However, the rarity of the disease results in reduced routine of treating physicians which can be illustrated by a high level of clinical practice variation. The rarity of diseases, such as AAV, is also a barrier for large, high-quality studies to establish high grade evidence to support clinical practice,[9,10] despite joint international efforts to conduct large randomised controlled trials.[11-19] To overcome this, physicians are supported by the development of clinical practice guidelines (CPGs), which are a common way to improve health care quality and safety through standardisation.[20] Indeed, CPGs are 'systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances'.[21] CPGs are known to improve clinical outcomes, promote consistency of care and reduce unwanted variation in health care as well as health care costs.[22-25] Developing CPGs for a rare disease like AAV is difficult because clinical evidence is based on only a few large, controlled clinical trials supplemented by mostly uncontrolled trials with smaller sample sizes, increased heterogeneity and limited generalisability.[9,24] Altogether, these limitations to the development of CPGs permit, and sometimes even encourage, practice variation within the care for a rare and complex disease like AAV. In order to improve the implementation of currently available CPGs on AAV in the Netherlands, a national initiative was launched to assemble and summarise expert opinion recommendations from a broad number of clinicians experienced in diagnosing and treating AAV patients.

The present study describes the results of a national initiative in the Netherlands that considered the challenges clinicians face when caring for their patients with AAV. Based on a longstanding, national collaboration in the field of AAV that previously resulted in a Dutch guideline on the diagnostics of small-vessel vasculitis,[26] we now developed consensus-based recommendations on the implementation of (inter)national guideline recommendations concerning diagnosis, terminology, and treatment (algorithms) for patients with AAV.

METHODS

ARCH foundation

In 2017, the Arthritis Research and Collaboration Hub (ARCH) foundation was initiated with the goal to improve healthcare for patients with rare systemic autoimmune diseases including AAV, by spreading expertise and by sharing medical information easily among physicians. With the support of ARCH, we initiated a national initiative to achieve consensus on diagnosing and treating AAV patients. The national initiative encompassed different methods and platforms to discuss and measure consensus guided by recommendations from current (inter)national guidelines.

A national working party for AAV was organised, which included experts in multiple disciplines as well as representatives from the Dutch Vasculitis Foundation and national professional associations including internal medicine, nephrology, rheumatology, clinical immunology, pathology, dermatology, otorhinolaryngology, and ophthalmology. The working group was led by core members: three medical specialists, a nephrologist (YKOT), rheumatologist (SWT), clinical immunologist (AR); the chairman of the Dutch Vasculitis Foundation (PMJV); and one physician-scientist (ED). Core members were responsible for organisation of meetings, leading discussions, and composition of the implementation document.

Implementation document

Between February and August 2018, an implementation document was composed by the multidisciplinary working group AAV. This document describes the basic approach to the diagnosis and management of AAV based on recently published management guidelines and clinical trials over the last two decades. Sixty-five recommendations from the following guidelines were used as basis for the implementation document: The Canadian Vasculitis research network (CanVasc) recommendations for the Management of Antineutrophil Cytoplasm Antibody-associated Vasculitides; the British Society of Rheumatology (BSR) and British Health Professionals in Rheumatology (BHPR) guideline for the management of adults with ANCA-associated vasculitis; and the European League Against Rheumatism/European Renal Association—European Dialysis and Transplant

Association (EULAR/ERA-EDTA) recommendations for the management of ANCA-associated vasculitis.[6-8] A recently developed Dutch guideline for ANCA-associated vasculitis with renal involvement was also consulted during this process because it encompassed the clinicians' preferences within the Dutch nephrology community.[6-8,27] All clinical trials and studies relevant to this implementation document were referenced in the implementation document. The document is made available as supplementary material.

Invitational consensus conference

An invitational consensus conference was organised where AAV patients, clinicians with experience on the treatment of AAV patients, paramedics, and physician-scientists in the field of AAV were invited through above-mentioned professional associations. Professional associations were responsible for the national distribution via their respective websites, scientific committees/representatives, or mailings. The Dutch Vasculitis Foundation was also invited. Additionally, members of the Dutch national working party on systemic autoimmune diseases (Systemische Autoimmuunziekten Nederland, SANL) were invited and the conference was advertised via the ARCH website. Recommendations from the implementation document were distributed in a timely manner to participants before the conference and discussed plenary. There was a focus on domains in the diagnosis and treatment of AAV where no clear consensus could be deducted from current (inter)national guidelines, or domains which were perceived as deviating from Dutch clinical practice or with a high clinical practice variation.

Online survey

An online survey was distributed nationally to all members of the two most represented professional organisations during the invitational conference: the Dutch Federation for Nephrology (NfN – 424 physicians) as part of the Dutch Society of Internal Medicine and Dutch Society for Rheumatology (NVR – 395 physicians). The online survey requested the opinion on statements covering all the domains of diagnosing and treating AAV.

Analysis

To achieve consensus on a national level, the Delphi method was used as basis for consecutive meetings and surveys as described. At any stage during the procedure, statements that were discussed and presented were voted upon by physicians on a binary scale (1: Agree [A] and 2: disagree [D]). Cut-off for consensus was set at 70% (dis)agreement. Descriptive statistics was used to summarise characteristics of the participants in different platforms and the degree of consensus per statement and domain. All analyses were performed with IBM SPSS Statistics version 23.

RESULTS

A total of 98 Dutch physicians participated in a Delphi procedure to discuss and find consensus during conference or online survey. Among these physicians were 75 nephrologists, 16 rheumatologists, 5 clinical immunologists, 1 pulmonologist, and 1 pathologist.

The criterium for consensus was met for 37 of 50 statements (74%). All statements with their level of agreement are documented in the implementation document (Supplementary material), including the contribution of a maximum of 10 patients.

For the sake of clarity and conciseness, a summary on the statements deemed most relevant to Dutch clinical practice in the domains of diagnostics, terminology, and (concomitant) treatment (table 1) are described in this manuscript.

Implementation domain: Diagnosis of AAV

Statement 1: High-quality antigen-specific immunoassay tests are recommended for ANCA testing

The previous consensus statement recommended that positive ANCA tests (generally performed through antigen-specific immunofluorescence) should be confirmed in a second independent test.[28] Implementation of this recommendation is traditionally left to the discretion of local practices and it is unclear whether the confirmation test should be performed and reported sequentially or simultaneously. Recently, a consensus statement by clinical immunologists was published on the basis of novel research recommending a high-quality antigen-specific immunoassay as the first test and confirmation by a second high-quality immunoassay (preferably by a second antigen-specific immunoassay test or an immunofluorescence test using neutrophils as substrates). Consequences of the implementation of this diagnostic strategy was extensively reviewed.[29] Consensus was unanimous (A: 100%, D: 0%) that high-quality antigen-specific immunoassay tests should be used instead of immunofluorescence for the assessment of patients with clinical suspicion of AAV. This was predominantly due to the superior sensitivity and specificity of antigen-specific immunoassays over immunofluorescence tests using neutrophils as substrates.[29,30]

ANCA testing should only be performed when there is clinical suspicion for AAV. The following key symptoms were commonly considered as supportive of clinical suspicion for AAV: bloody crusts in the nose (93%), lung nodules (90%), (epi)scleritis/retro-periorbital inflammation (89%), arthritis/arthralgia (87%), renal insufficiency, (microscopic) haematuria and/or proteinuria (84%), fever of unknown origin (81%), skin manifestations (80%), and neuropathy (80%). When AAV is suspected, initial diagnostic evaluation should include: urinary sediment

Table 1. *Recommendations*

No.	Recommendation statements	Level of agreement (LoA, %)
Diagnosis		
1	For adequate ANCA diagnosis in the context of a clinical suspicion of ANCA-associated vasculitis, screening should be done with antigen-specific tests for MPO and PR3-ANCA; the ANCA IIF test can be left out.	100
2a	In case of clinical suspicion of AAV and a high-quality, high-positive ANCA test, histopathological evidence is necessary for the diagnosis AAV.	33
2b	In case of clinical suspicion of AAV and a high-quality, high-positive ANCA test, histopathological evidence is highly desirable for AAV treatment.	89
Terminology		
3	In all AAV patients, a distinction is made between generalised disease and non-generalised disease.	99
4	In patients treated for AAV, remission is determined using the following terms: 'clinical remission', 'remission under therapy' or 'medication-free remission' with the aim of controlling the transition from induction to maintenance therapy and possible discontinuation of therapy.	72
Treatment		
5	Cyclophosphamide and/or rituximab are both effective in the treatment of a newly diagnosed patient with generalised disease	LoA: n.a. PP for CYCpo/CYCiv/RTX: 66/21/13
6	Plasma exchange should be performed in patients with life-threatening disease.	70
7a	Azathioprine is the most preferred agent for maintenance therapy in addition to low-dose oral steroids	LoA: n.a. PP for AZA/CYC/LEF/MTX/ MMF/RTX/ Anti-TNF: 86/0/0/5/2/7/0
7b	In AAV patients who received rituximab as induction therapy, one may consider treatment without maintenance therapy (i.e. reducing prednisolone to 0 mg/day).	71
8	The duration (2-4 years) of maintenance therapy is stratified on the basis of risk factors for relapse.	86
Concomitant treatment		
9	In case of suspicion of AAV with ENT involvement, S. aureus carriage should be determined.	76
10	All patients with generalised AAV on remission induction therapy should receive prophylactic treatment against PCP.	92
11	PCP prophylaxis is indicated until intensive immunosuppression is tapered to a safe, low dosage.	LoA: n.a. PP for < 5mg, < 7.5, < 10, < 15; < 20; CYCstop; other: 8/8/18/2/6/52/5
12	Mesna should not be used in all patients receiving cyclophosphamide.	92
Treatment algorithm		
13	Do you agree with the proposed treatment algorithm?	73

AAV = ANCA-associated vasculitis; ANCA = anti-neutrophil cytoplasmic antibody; anti-TNF = anti-tumour necrosis factor; AZA = azathioprine; CYCiv = intravenous cyclophosphamide; CYCpo = oral cyclophosphamide; ENT = ear-nose-throat; GPA = granulomatosis with polyangiitis; IIF = indirect immunofluorescence; LEF = leflunomide; LoA: level of agreement; MPA = microscopic polyangiitis; MMF = mycophenolate mofetil; MPO = myeloperoxidase; MTX = methotrexate; n.a. = not applicable; PCP = Pneumocystis Pneumonia; PP = physicians' preference; PR3 = proteinase 3; RTX = rituximab; S. aureus = *Staphylococcus aureus*

(100%), ANCA serology (98%), renal function (98%), chest X-ray (94%), inflammation markers (92%), and complete blood count (87%). There was no consensus on the added value of electrolytes (49%) and liver enzymes (48%). Histopathological evidence (30%) was not required during initial evaluation of patients, as further discussed in Statement 2.

Statements 2a and b: Histopathological evidence of small-vessel vasculitis is highly desirable, but lack thereof should not interfere with the initiation of adequate treatment

There is a long-standing debate as to whether a kidney biopsy, involving a high-risk procedure, is indicated when there is already strong clinical suspicion of AAV with renal insufficiency, an active urine sediment with dysmorphic erythrocyturia, proteinuria, and a positive ANCA test. It has been shown that in such cases the chance for a kidney biopsy to confirm ANCA-associated glomerulonephritis lesions is probably well above 90%.[31] A recent study summarising the outcomes of histopathological examinations of non-renal biopsies in GPA patients demonstrated typical vasculitis findings in only 39% of the biopsies, while 55% of the biopsies showed non-specific lesions.[32] Consequently, the absence of specific histopathological lesions in such cases requires serious reconsideration of the clinical diagnosis of AAV.

Consensus was reached on the importance of obtaining histopathological evidence for vasculitis from an affected organ (e.g., kidney, lung, nose, skin) in particular, to support treatment choices in those cases that require initiation of intensive remission induction therapy. There was no majority found to make a distinction for myeloperoxidase (MPO) and proteinase-3 (PR3)-positive patients within this particular discussion on obtaining histopathological evidence of vasculitis. Because the recent study by Bossuyt et al. showed only small differences in sensitivity of PR3 (94%) vs. MPO (87%) and specificity of PR3 (90%) vs. MPO (83%), it would not affect the wording 'highly desirable' within this statement.[29] In general, any diagnostic procedure should be performed when it has added value for diagnosis or consequences for treatment decisions and/or prognosis in AAV patients. Especially in AAV patients with renal involvement, a kidney biopsy will often provide answers that are important for diagnosis, treatment intensity, and prognosis of AAV disease and therefore the rationale to perform a renal biopsy stands. However, as for any invasive diagnostic test, the risks of a kidney biopsy should be considered (i.e., 3.5% bleeding of which 0.9% need transfusion and 0.6% need angiographic intervention; up to 14% AV-fistulas; ≤ 0.01% nephrectomy).[33,34]

Histopathological validation of the diagnosis AAV is of particular importance when adequate clinical response is unexpectedly lacking after initiation of treatment. In patients without renal symptoms, the potential clinical value of a kidney biopsy to confirm AAV remains unknown.

Implementation domain: Terminology

Statements 3 and 4: Homogenous terminology of disease severity and disease states support therapeutic decision-making

Guidelines have used different terminology to define disease severity of AAV requiring less or more intensive immunosuppressive treatment. Definitions of disease stages formulated by the EUVAS group (European Vasculitis Study Group) and the WGET research group (Wegener's Granulomatosis Etanercept Trial Research Group) are being used frequently to classify AAV in clinical trials and guidelines.[35,36] EUVAS defines four different disease stages: localised, early systemic, generalised, and severe AAV. Localised AAV is characterised by ear-nose-throat (ENT) and lung involvement with mild renal impairment (serum creatinine < 120 umol/l). In cases of other non-upper respiratory tract organ involvement on top of localised disease, it is called early systemic disease. The generalised form is defined as organ-threatening disease in organs outside the ENT and lungs with a serum creatinine of < 500 umol/l. When the serum creatinine level is > 500 umol/l, it is called severe disease. WGET makes a distinction between limited and severe disease. Limited disease is defined as sinus, skin, joints, and mild renal manifestations. Life- or organ-threatening manifestations, such as rapidly progressive glomerulonephritis, pulmonary haemorrhage and vasculitis neuropathy is defined as severe AAV. On the basis of these disease stages, different treatment regimens are recommended (more severe stages require more intensive immunosuppressive medication).

It is clear that for implementation of guideline recommendations, consensus is needed on terminology. As such, consensus was reached on differentiating generalised disease from life-threatening and non-generalised disease (A: 99%, D: 1%). Generalised disease is defined as organ-threatening disease/involvement of organs such as kidneys, lungs, heart, and peripheral or central nervous system, whereas life-threatening disease includes rapidly progressive glomerulonephritis leading to end-stage renal disease, severe pulmonary haemorrhage, and/or dual anti-glomerular basement membrane (GBM) and ANCA positivity. Non-generalised disease is when none of the above-mentioned symptoms are present. Intensity of immunosuppression and treatment approach was coupled to this terminology.[37]

Also, consensus was reached on two definitions of clinical remission ('remission on therapy' and 'medication-free

remission') (A: 72%, D: 28%), which also was coupled to treatment decision regarding maintenance therapy. 'Remission on therapy' is the state of clinical remission ≥ 6 months at a prednisolone dose ≤ 10 mg/day and medication-free remission is clinical remission without any immunosuppressive medication. From these disease states, it also becomes obvious that 'clinical response' to treatment is a clinical state where an improvement of disease activity is observed but 'clinical remission' is not yet achieved.

Implementation domain: Treatment of AAV

Statement 5: Cyclophosphamide and/or rituximab are both effective in the treatment of a newly diagnosed patient with generalised disease

Several guidelines recommend glucocorticoids in combination with cyclophosphamide (CYC) or rituximab (RTX) as remission induction therapy for generalised and life-threatening disease.[6-8] Oral CYC was introduced in the 1960s as a treatment for AAV and significantly changed the disease course. Unfortunately, CYC treatment is accompanied by serious side effects and (long-term) toxicities in association to its life-long, cumulative dose. Significant progression has been made by studies investigating lower dosing of CYC without loss of efficacy: the CYCLOPS study showed that pulsed intravenous CYC was non-inferior to daily oral CYC to achieve remission, long-term survival, preservation of renal function, and prevention of end-stage renal failure. Equivalence on these hard endpoints were observed despite an increased relapse rate in patients treated with intravenous CYC. At the same time, intravenous CYC was associated with fewer side effects.[11] Of note, several studies[11,38-40] have shown that the risk for relapse is inversely associated with the cumulative CYC dose received during the induction phase. In 2010, the RAVE study compared RTX to oral CYC demonstrating that RTX was non-inferior to CYC in achieving remission in newly diagnosed AAV patients with generalised disease; of note, RTX was superior to CYC in relapsing AAV patients.[18]

Overall, CPGs have remained careful in their recommendations on a preferable agent for remission induction therapy and recommend either CYC or RTX in AAV patients with generalised disease, and azathioprine (AZA), methotrexate (MTX), or mycophenolate mofetil (MMF) in AAV patients with non-generalised disease unless contraindicated (i.e., impaired renal function). In the present initiative, we surveyed physicians' preference and found that in the Netherlands, 66% of physicians prescribe oral CYC, 21% intravenous CYC, and 13% RTX as induction treatment for AAV patients with generalised disease. Thus, we are aligned with the CPGs' recommendations, and have stated in the implementation document that CYC and RTX are equivalent therapy choices as induction treatment. Clinicians should consider the (dis)advantages of CYC and RTX for each individual patient who requires remission induction therapy.

Statement 6: Plasma exchange therapy should be performed in patients with life-threatening disease

In addition to immunosuppressive agents, the EULAR recommendations and BSR/BHPR guideline suggest to add plasma exchange (PLEX) therapy in patients with a serum creatinine level > 500 umol/l and/or pulmonary haemorrhage.[6,8] The CanVasc recommends PLEX only in patients who remain refractory with immunosuppressive therapy because of controversial long-term outcomes on renal survival and mortality.[7] Preliminary data of the PEXIVAS study, which was presented at the EULAR/ERA-EDTA congress, has shown that additional PLEX had no favourable long-term outcomes in comparison to regular immunosuppressive therapy, but these were not published at the time of this inventory. Until the results of this trial are published and/or confirmed, we recommend to perform PLEX in patients with life-threatening disease. Consensus was achieved on adding PLEX if the patient suffers from rapidly progressive glomerulonephritis and/or alveolar haemorrhage (A: 70%, D: 30%).

Statement 7a: Azathioprine is the most preferred agent for maintenance therapy

Statement 7b: After an RTX-based remission induction therapy, the physician may consider treatment without maintenance therapy (i.e., reducing prednisolone to 0 mg/day).

When remission is achieved after remission induction therapy, maintenance therapy is required to prevent relapses. Previous studies have shown that relapses occur in 30-50% of patients after reducing or discontinuing therapy.[41-44] In the last decade, pioneering clinical trials studying maintenance therapy drugs and duration have been conducted.[12,16,45] The CYCAZAREM study investigated the replacement of CYC by AZA after achieving remission in comparison to prolonged CYC treatment as maintenance therapy. No difference was observed in relapse rates between these groups, concluding that AZA is a safe alternative for maintenance therapy.[14] Hereafter, AZA has been considered the most effective treatment for maintaining clinical remission compared to MTX (WEGENT), MMF (IMPROVE), leflunomide (LEM), belimumab (BREVAS), or anti-TNF (WGET).[13,17,36,46,47] Most recently, the MAINRITSAN studies[12,45,48] demonstrated the superiority of RTX maintenance treatment over AZA, in which case fixed re-treatment every six months or tailored RTX infusions on the basis of CD19+ cells and ANCA levels had comparable efficacy on maintaining clinical remission. However, these data were not publicly available at the time of the survey.

In CPGs, after remission induction therapy, the CanVasc group and the BSR/BHPR guideline recommend to use either AZA, MTX, or RTX as maintenance therapy and if not tolerated, MMF or leflunomide.[6,7] According to the EULAR recommendations, the aforementioned agents AZA, RTX, MTX, and MMF can be used as maintenance therapy.[8] Besides the RAVE study, there is a lack of data on maintenance treatment after remission induction therapy with RTX. This study confirmed that no maintenance treatment after RTX was equivalent to AZA maintenance after CYC.[18,49] Consequently, recommendations on optimal maintenance therapy remained unclear.

In the present study, we surveyed physicians' preference and found that AZA is the most preferred maintenance therapy in addition to low-dose oral steroids (86%) in the Netherlands. RTX maintenance was only given by 7% of physicians. Upon RTX given as remission induction therapy, 71% of physicians would consider continuing without any maintenance therapy (similar to the RAVE study).[18,49] It needs to be mentioned that at the time of the survey, the long-term results of the MAINRITSAN-1 and MAINRITSAN-2 studies were not publicly available yet and might have influenced the formulation of this statement which was built upon physicians' preferences.

Statement 8: The duration of maintenance therapy should be based on risk factors for future relapse

There is no clear consensus on the optimal duration of maintenance therapy. In 2017, the EUVAS working group analysed in 380 new AAV patients whether duration of AZA maintenance influenced relapse rates at five years. Interestingly, discontinuing AZA maintenance ≤ 12 months results in significant more relapses, whereas this phenomenon disappeared in patients with AZA maintenance for 18 months or more.[50] Guidelines also recommend to continue maintenance therapy for 18-24 months after diagnosis.[6-8] A Dutch study demonstrated no additional value of extending maintenance therapy (4 years vs. 1 year) in AAV patients, however unfortunately, the study was prematurely stopped due to slow patient recruitment.[51] More recently, a large randomised, controlled trial (REMAIN study) demonstrated a significant reduction of relapse risk when maintenance therapy with low-dose steroids and AZA was maintained for four years compared to two years.[16] Despite these conflicting results and well-established risk factors for relapse derived from several large RCTs, as detailed in the implementation document, table 6 (Supplementary material),[44,52-54] consensus was reached on the recommendation that risk factors for relapse should be taken into account at the time of discontinuing maintenance therapy after at least two years (A: 86%, D: 14%).

Implementation domain: Concomitant treatment

Statement 9: Nasal carriage of Staphylococcus aureus should be determined in AAV patients with ear-nose-throat involvement

Previous studies have shown that chronic carriage of nasal *Staphylococcus aureus* (≥ 75% of the cultures were positive) in patients with nasal disease is associated with higher relapse rates and treatment with trimethoprim/sulfamethoxazole 960 mg given twice daily for two years is able to reduce relapse rates.[55,56] In the AAV guidelines, no statement or recommendation is made on testing nasal carriage of *S. aureus*, but only on treating this condition. Although in the general population approximately one-third has intermittent and one-third has chronic carriage of *S. aureus*, 60-70% of GPA patients are carriers of *S. aureus*.[57] We reached consensus on the need for determining nasal carriage of *S. aureus* in patients with (a clinical suspicion of) AAV and ENT involvement (A: 76%, D: 24%). In carriers, treatment with oral trimethoprim/sulfamethoxazole can be considered.

Statement 10: All AAV patients on remission induction therapy should be treated with pneumocystis pneumonia (PCP) prophylaxis

Statement 11: PCP is indicated until intensive immunosuppression is tapered to a safe, low dosage

Patients suffering from autoimmune diseases and receiving immunosuppressive treatment are at increased risk of developing pneumocystis pneumonia (PCP).[58-60] Several papers describe an important role for corticosteroids in the development of PCP, whether or not in combination with other cytotoxic agents.[58,61-63] The use of PCP prophylaxis is advised in patients with corticosteroids ≥ 20 mg/day for at least one month and is especially recommended in the presence of additional T-cell defects or cytotoxic agents such as CYC.[64]

Guidelines have stated that PCP prophylaxis should be given to patients on remission induction therapy, if not contraindicated. The EULAR/ERA-EDTA recommends prophylaxis against PCP only in patients receiving CYC; there is no recommendation for patients receiving RTX.[8] The BSR/BHPR guideline briefly mentions that PCP prophylaxis should be considered for AAV patients on immunosuppressive therapy[6] and the CanVasc group recommends prophylaxis against PCP in patients receiving either CYC or RTX. In patients receiving CYC, prophylaxis should be continued for at least three months after cessation of CYC, because of the occurrence of PCP infections in several case reports after withdrawal of remission induction therapy.[61] There is no statement on the optimal duration of PCP prophylaxis after remission induction treatment with RTX.[7] Based on two different studies, PCP prophylaxis seems to be indicated in RTX-treated AAV patients because of severe infections.[65,66]

We surveyed physicians' preference regarding PCP prophylaxis and found that in the Netherlands, 92% of clinicians will prescribe PCP prophylaxis during remission induction therapy; 52% would stop prophylactic treatment for PCP simultaneously with tapering or termination of CYC; 18% would discontinue PCP prophylaxis at the time of reaching a prednisolone dose of ≤ 10 mg/day, 6% when reaching ≤ 20 mg/day, 2% at reaching ≤ 15mg/day, 8% when reaching ≤ 7.5 mg/day, and 8% when reaching ≤ 5 mg/day.

Statement 12: Mesna should not be used in all patients receiving cyclophosphamide

High-dose intravenous CYC or long-term oral CYC (> 3 months) is associated with haemorrhagic cystitis and bladder cancer due to the interaction between the acrolein metabolite of CYC and the bladder wall.[67,68] Mesna (2-mercaptoethane sulfonate) is able to inactivate acrolein and prevent these side effects.[69] These results are based on CYC use in patients with cancer receiving higher doses of CYC in comparison to patients with rheumatic diseases. Because of the lack of evidence in patients with rheumatic diseases, there are no strong recommendations for mesna use in guidelines for AAV. A national position statement by Dutch rheumatologists has advised that mesna should only be considered in patients with long-term CYC use and at high risk for bladder toxicity (e.g., disturbed bladder emptying, urinary retention, or recurrent cystitis).[6-8,67,70] Structural examination of the urine sediment helps to detect bladder toxicity.[27,67,70] Indeed, consensus was reached

Figure 1. *Treatment algorithm for AAV*

* Non-generalised disease: different from 'generalised disease' or 'life-threatening disease'
** Generalised disease: organ-threatening disease/ involvement of organs such as kidneys, lungs, heart, and peripheral or central nervous system
*** Life-threatening disease: rapidly progressive glomerulonephritis leading to end-stage renal disease; severe pulmonary haemorrhage; dual anti-GBM and ANCA positivity
¶The treating physician should decide whether treatment with methylprednisolone i.v. is required. For patients with a mildly progressive course, oral prednisolone may be sufficient.
AAV = ANCA-associated vasculitis; AZA = azathioprine; CYC = cyclophosphamide; i.v. = intravenous; MMF = mycophenolate mofetil; MP = methyl prednisolone; MTX = methotrexate; PLEX = plasma exchange; RTX = rituximab

that mesna should not be prescribed in all CYC-treated AAV patients (A: 91%, D: 9%).

Statement 13: Treatment algorithm – figure 1
Based on several statements discussed above and detailed in the implementation document (Supplementary material) consensus was reached on a treatment algorithm (A: 73%, D: 27%) for AAV patients (figure 1).

Categorisation of AAV patients upon clinical presentation
Delay in diagnosing or initiating adequate therapy in patients with severe AAV may have harmful consequences. Therefore, the treatment algorithm first necessitates at an early stage, the categorisation of patients by consensus-based definitions, i.e., 'life-threatening disease', 'generalised disease' or 'non-generalised disease'.

Associated with disease categories is the choice of remission induction therapy:
If there is a life-threatening disease, consensus was that PLEX therapy should be considered in addition to treatment with intravenous methylprednisolone pulse therapy and cyclophosphamide whether or not combined with RTX.[15,71] Of note, agreement on the use of methylprednisolone pulses was found on the background that PLEX therapy is not readily available in all Dutch hospitals. As mentioned before, publication of the results from the PEXIVAS study will undoubtedly improve the selection criteria for which AAV patients PLEX therapy can be indicated. In patients with generalised disease, either intravenous methylprednisolone or oral prednisolone can be chosen in combination with CYC or RTX during the remission induction phase. In specific cases (e.g., patients with a mildly progressive course), oral prednisolone combined with an immunosuppressive agent may be sufficient. Patients with non-generalised disease should start with high doses of prednisolone combined with an immunosuppressive agent such as MTX or MMF during the remission induction phase.

Categorisation of AAV patients when responding to treatment:
Usually, patients achieve clinical remission, defined as absence of disease activity (BVAS score of 0), between 3-6 months after initiation of remission induction therapy. At this state, patients are 'in remission on therapy'. At that time, maintenance therapy should be initiated to prevent disease relapses and is interdependent to the chosen remission-induction treatment. As such, patients with life-threatening disease should continue with maintenance therapy in the form of low-dose oral prednisolone combined with AZA, RTX, or MTX. The choice for a specific agent depends on the tolerance of a patient and the preference of the treating physician. Patients with generalised disease treated with CYC as remission-induction will continue prednisolone combined with AZA, RTX, or MTX. Patients with generalised disease treated with RTX as remission-induction will taper prednisolone to 0 mg/day and stop maintenance therapy, unless the patient suffers from relapsing disease. In that case, maintenance therapy should be continued with low-dose oral prednisolone combined with AZA, RTX, or MTX. Patients with non-generalised disease should continue treatment with low-dose oral prednisolone in combination with MTX or MMF.
The ultimate aim is to achieve 'medication-free remission'. This goal can be realised within two to four years after starting AAV treatment, where the duration of maintenance treatment should be guided by the individual patient's risk classification for a relapse.

DISCUSSION

AAV is a complex, systemic autoimmune disease with a low disease prevalence and therefore intrinsically difficult to diagnose and treat, which is eventually reflected in clinical practice variation. Due to the rarity of the disease, it is challenging to obtain high-quality clinical evidence to underpin firm and coherent recommendations in guidelines for clinicians allowing, intended or not, for considerable differences in clinical AAV management between physicians. In this setting, the present study described the development of a national, multidisciplinary, consensus-based implementation document. By means of a nation-wide consensus the implementation of guideline recommendations can be improved because clear and practical guidance is given for treating physicians, including for those issues that cannot be addressed by guidelines due to lack of data or adequate, comparative studies. Moreover, a consensus-based implementation document can address practice variation and thereby improve physicians collective experience with a uniform management of patients with a rare and severe disease like AAV. Ultimately, harmonisation of the management will improve standardised evaluation of care for AAV patients which is a prerequisite for improving care in the future.

We described a Dutch national implementation document on the basis of previously published guidelines and consensus (of ≥ 70%) among nation-wide healthcare professionals experienced in treating AAV patients. This implementation document is complementary to current evidence-based guidelines because it enabled us to provide recommendations on practical issues where evidence is not readily available. Exemplary are the recommendations on terminology annotating disease subsets, disease states, and disease extent in direct relation to treatment choices;

the methods of ANCA testing and confirmation; the minimal requirements for the organisation of care around AAV patients; and the use of PLEX in AAV patients with life-threatening disease. For the latter, it is important to note that the level of agreement on the indications for PLEX can be influenced in the near future by the publication of the PEXIVAS study results and the results of a meta-analysis of all studies on PLEX in AAV collectively. As such, this implementation document with high rates of consensus facilitates the harmonisation of local treatment protocols for AAV and reduce practice variation with the intent to improve care for AAV patients nationwide.

Not unexpectedly, we encountered several noteworthy findings during this study. First, based on the characteristics of responders on our nation-wide invitation through professional associations, the majority of the participants were nephrologists and to a lesser extent, rheumatologists. It is therefore important to note that due to a higher representation of nephrologists, one can argue that this document is based on a consensus between nephrologists rather than the broad concept of any treating physician involved in the treatment of AAV. However, although speculative at this time, participation to our study indicates that AAV patients in the Netherlands are in general, treated by nephrologists and/or rheumatologists. To confirm this observation, a more in-depth study at the individual patient-level is needed. Second, a remarkable observation was the high frequency (66%) of participants employing oral CYC as the preferable first-line therapy, while several trials and guidelines, including Dutch guidelines, recommend intravenous CYC or RTX because of reduced toxicity.[6-8,11,18] One can only speculate on the rationale of individual physicians however, possible explanations can be found in the suggestion of reduced relapse rates in the pivotal CYCLOPS study, the convenience of oral CYC administration without the need of hospital admissions for intravenous treatments (i.e., CYC iv or RTX), and its lower costs. Third, the preference for AZA as maintenance therapy in the majority of the physicians is noteworthy. At the time of this study, the long-term results of the MAINRITSAN-1 and MAINRITSAN-2 studies were not widely available.

Altogether, we present an implementation document for the diagnosis and treatment of AAV that is complementary to a previously published Dutch multidisciplinary guideline on the diagnosis of small-vessel vasculitis[26] and the Dutch guideline for treatment of renal vasculitis.[27] The described implementation strategy can be exemplary for other countries to translate international guideline recommendations into common clinical practice. In addition, during our study, several clinically-relevant issues were identified for the AAV research agenda, such as the value of risk-stratification in deciding whether to stop treatment and the position of rituximab as maintenance treatment tailored by immunological parameters (i.e., ANCA and B-cell levels). Future studies will be directed at evaluating whether this consensus-based, implementation strategy reduces clinical practice variation in the Netherlands and improves healthcare for AAV patients. To do so, a nation-wide study has been started to evaluate the care provided to AAV patients in the past 10 years. This study will provide important insights into current practice variation in the Netherlands with regard to diagnosis (including in-hospital time-to-diagnosis and patient characteristics), treatment regimens (including immunosuppressants used for induction and maintenance therapy and duration of treatment), and disease outcomes (including mortality, infections, and malignancies); at the same time, indicators for the quality of care can be defined. Also, in order to contain practice variation, strategies will be developed to improve access to expert advice and/or consultation. Ultimately, these efforts will lead to the improvement of care and disease amelioration for AAV patients.

ACKNOWLEDGMENTS

The work of E. Dirikgil., Y.K.O. Teng, S.W. Tas, P.M.J. Verhoeven, A. Rutgers, J.M. van Laar, and H.J. Bernelot-Moens is financially supported by Arthritis Research and Collaboration Hub (ARCH). Y.K.O. Teng is funded by the Dutch Kidney Foundation (KJPB12.028), Clinical Fellowship from the Netherlands Organization for Scientific Research (90713460).

We thank all patients and professional healthcare providers who participated in the consensus meeting and online survey for their effort and time.

The final implementation document is published in Dutch at the website of ARCH: https://www.arch.nl/wp-content/uploads/2018/10/ConsensusdocAAV-1.pdf

SUPPLEMENTARY MATERIAL

For the implementation document in Dutch, please request via email: y.k.o.teng@lumc.nl or info@arch.nl

REFERENCES

1. Jennette JC. Overview of the 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. Clin Exp Nephrol. 2013;17:603-6.
2. Walton EW. Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). Br Med J. 1958;2:265-70.
3. Smith RM. Update on the treatment of ANCA associated vasculitis. Presse Med. 2015;44:e241-9.

4. Tan JA, Dehghan N, Chen W, Xie H, Esdaile JM, Avina-Zubieta JA. Mortality in ANCA-associated vasculitis: ameta-analysis of observational studies. Ann Rheum Dis. 2017;76:1566-74.
5. Kallenberg CG. Key advances in the clinical approach to ANCA-associated vasculitis. Nat Rev Rheumatol. 2014;10:484-93.
6. Ntatsaki E, Carruthers D, Chakravarty K, et al. BSR and BHPR guideline for the management of adults with ANCA-associated vasculitis. Rheumatology (Oxford). 2014;532306-9.
7. McGeoch L, Twilt M, Famorca L, et al. CanVasc Recommendations for the Management of Antineutrophil Cytoplasm Antibody-associated Vasculitides. J Rheumatol. 2016;43:97-120.
8. Yates M, Watts RA, Bajema IM, et al. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. Ann Rheum Dis. 2016;75:1583-94.
9. Rath A, Salamon V, Peixoto S, et al. A systematic literature review of evidence-based clinical practice for rare diseases: what are the perceived and real barriers for improving the evidence and how can they be overcome? Trials. 2017;18:556.
10. Wennberg JE. Unwarranted variations in healthcare delivery: implications for academic medical centres. BMJ. 2002;325:961-4.
11. de Groot K, Harper L, Jayne DR, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. Ann Intern Med. 2009;150:670-80.
12. Guillevin L, Pagnoux C, Karras A, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. N Engl J Med. 2014;371:1771-80.
13. Hiemstra TF, Walsh M, Mahr A, et al. Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial. JAMA. 2010;304:2381-8.
14. Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med. 2003;349:36-44.
15. Jayne DR, Gaskin G, Rasmussen N, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol. 2007;18:2180-8.
16. Karras A, Pagnoux C, Haubitz M, et al. Randomised controlled trial of prolonged treatment in the remission phase of ANCA-associated vasculitis. Ann Rheum Dis. 2017;76:1662-8.
17. Pagnoux C, Mahr A, Hamidou MA, et al. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. N Engl J Med. 2008;359:2790-803.
18. Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med. 2010;363:221-32.
19. De Groot K, Rasmussen N, Bacon PA, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2005;52:2461-9.
20. Kurtin P, Stucky E. Standardize to excellence: improving the quality and safety of care with clinical pathways. Pediatr Clin North Am. 2009;56:893-904.
21. Clinical Practice Guidelines: Directions for a New Program. In: Field MJ, Lohr KN, editors. Clinical Practice Guidelines: Directions for a New Program. Washington (DC)1990.
22. Berwick DM. Disseminating innovations in health care. JAMA. 2003;289:1969-75.
23. Morris ZS, Wooding S, Grant J. The answer is 17 years, what is the question: understanding time lags in translational research. J R Soc Med. 2011;104:510-20.
24. Woolf SH, Grol R, Hutchinson A, Eccles M, Grimshaw J. Clinical guidelines: potential benefits, limitations, and harms of clinical guidelines. BMJ. 1999;318:527-30.
25. Kristensen N, Nymann C, Konradsen H. Implementing research results in clinical practice- the experiences of healthcare professionals. BMC Health Serv Res. 2016;16:48.
26. Thio HB, Balak DM, Meilof JF, Stegeman CA, Voskuyl AE, Consensus diagnostic small-vessel vasculitis W. [Guideline 'Diagnostics of small-vessel vasculitis']. Ned Tijdschr Geneeskd. 2012;156:A4317.
27. Remmelts HHF, Dam Bv, Douma CE. ANCA-geassocieerde vasculitis met renale betrokkenheid. Nederlandse federatie voor Nefrologie. 2018.
28. Savige J, Gillis D, Benson E, et al. International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). Am J Clin Pathol. 1999;111:507-13.
29. Bossuyt X, Cohen Tervaert JW, Arimura Y, et al. Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. Nat Rev Rheumatol. 2017;13:683-92.
30. Damoiseaux J, Csernok E, Rasmussen N, et al. Detection of antineutrophil cytoplasmic antibodies (ANCAs): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. Ann Rheum Dis. 2017;76:647-53.
31. Aasarod K, Bostad L, Hammerstrom J, Jorstad S, Iversen BM. Renal histopathology and clinical course in 94 patients with Wegener's granulomatosis. Nephrol Dial Transplant. 2001;16:953-60.
32. Masiak A, Zdrojewski Z, Peksa R, et al. The usefulness of histopathological examinations of non-renal biopsies in the diagnosis of granulomatosis with polyangiitis. Reumatologia. 2017;55:230-6.
33. Hogan JJ, Mocanu M, Berns JS. The Native Kidney Biopsy: Update and Evidence for Best Practice. Clinical journal of the American Society of Nephrology: CJASN. 2016;11:354-62.
34. Corapi KM, Chen JL, Balk EM, Gordon CE. Bleeding complications of native kidney biopsy: a systematic review and meta-analysis. Am J Kidney Dis. 2012;60:62-73.
35. Hellmich B, Flossmann O, Gross WL, et al. EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on anti-neutrophil cytoplasm antibody-associated vasculitis. Ann Rheum Dis. 2007;66:605-17.
36. Wegener's Granulomatosis Etanercept Trial Research G. Etanercept plus standard therapy for Wegener's granulomatosis. N Engl J Med. 2005;352:351-61.
37. European therapeutic trials in ANCA-associated systemic vasculitis: disease scoring, consensus regimens and proposed clinical trials. European Community Study Group on Clinical Trials in Systemic Vasculitis ECSYSVASTRIAL. Clin Exp Immunol. 1995;101 Suppl 1:29-34.
38. Koldingsnes W, Nossent H. Predictors of survival and organ damage in Wegener's granulomatosis. Rheumatology (Oxford). 2002;41:572-81.
39. Neumann I, Kain R, Regele H, Soleiman A, Kandutsch S, Meisl FT. Histological and clinical predictors of early and late renal outcome in ANCA-associated vasculitis. Nephrol Dial Transplant. 2005;20:96-104.
40. Guillevin L, Cohen P, Mahr A, et al. Treatment of polyarteritis nodosa and microscopic polyangiitis with poor prognosis factors: a prospective trial comparing glucocorticoids and six or twelve cyclophosphamide pulses in sixty-five patients. Arthritis Rheum. 2003;49:93-100.
41. Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener granulomatosis: an analysis of 158 patients. Ann Intern Med. 1992;116:488-98.
42. Reinhold-Keller E, Beuge N, Latza U, et al. An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. Arthritis Rheum. 2000;43:1021-32.
43. Westman KW, Bygren PG, Olsson H, Ranstam J, Wieslander J. Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. J Am Soc Nephrol. 1998;9:842-52.
44. Walsh M, Flossmann O, Berden A, et al. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2012;64:542-8.
45. Charles P, Terrier B, Perrodeau E, et al. Comparison of individually tailored versus fixed-schedule rituximab regimen to maintain ANCA-associated vasculitis remission: results of a multicentre, randomised controlled, phase III trial (MAINRITSAN2). Ann Rheum Dis. 2018;77:1143-9.
46. Metzler C, Miehle N, Manger K, et al. Elevated relapse rate under oral methotrexate versus leflunomide for maintenance of remission in Wegener's granulomatosis. Rheumatology (Oxford). 2007;46:1087-91.
47. Jayne D, Blockmans D, Luqmani R, et al. Efficacy and Safety of Belimumab and Azathioprine for Maintenance of Remission in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis: A Randomized Controlled Study. Arthritis Rheumatol. 2019;71:952-63.

48. Terrier B, Pagnoux C, Perrodeau E, et al. Long-term efficacy of remission-maintenance regimens for ANCA-associated vasculitides. Ann Rheum Dis. 2018;77:1150-6.

49. Specks U, Merkel PA, Seo P, et al. Efficacy of remission-induction regimens for ANCA-associated vasculitis. N Engl J Med. 2013;369:417-27.

50. de Joode AAE, Sanders JSF, Puechal X, et al. Long term azathioprine maintenance therapy in ANCA-associated vasculitis: combined results of long-term follow-up data. Rheumatology (Oxford). 2017;56:1894-901.

51. Sanders JS, de Joode AA, DeSevaux RG, et al. Extended versus standard azathioprine maintenance therapy in newly diagnosed proteinase-3 anti-neutrophil cytoplasmic antibody-associated vasculitis patients who remain cytoplasmic anti-neutrophil cytoplasmic antibody-positive after induction of remission: a randomized clinical trial. Nephrol Dial Transplant. 2016;31:1453-9.

52. Harper L, Morgan MD, Walsh M, et al. Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated vasculitis: long-term follow-up. Ann Rheum Dis. 2012;71:955-60.

53. Hogan SL, Falk RJ, Chin H, et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. Ann Intern Med. 2005;143:621-31.

54. Morgan MD, Szeto M, Walsh M, et al. Negative anti-neutrophil cytoplasm antibody at switch to maintenance therapy is associated with a reduced risk of relapse. Arthritis Res Ther. 2017;19:129.

55. Stegeman CA, Tervaert JW, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CG. Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. Ann Intern Med. 1994;120:12-7.

56. Stegeman CA, Tervaert JW, de Jong PE, Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. N Engl J Med. 1996;335:16-20.

57. Kallenberg CG, Tadema H. Vasculitis and infections: contribution to the issue of autoimmunity reviews devoted to "autoimmunity and infection". Autoimmun Rev. 2008;8:29-32.

58. Gerrard JG. Pneumocystis carinii pneumonia in HIV-negative immunocompromised adults. Med J Aust. 1995;162:233-5.

59. Roblot F, Godet C, Le Moal G, et al. Analysis of underlying diseases and prognosis factors associated with Pneumocystis carinii pneumonia in immunocompromised HIV-negative patients. Eur J Clin Microbiol Infect Dis. 2002;21:523-31.

60. Yale SH, Limper AH. Pneumocystis carinii pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. Mayo Clin Proc. 1996;71:5-13.

61. Arend SM, Kroon FP, van't Wout JW. Pneumocystis carinii pneumonia in patients without AIDS, 1980 through 1993. An analysis of 78 cases. Arch Intern Med. 1995;155:2436-41.

62. Godeau B, Coutant-Perronne V, Le Thi Huong D, et al. Pneumocystis carinii pneumonia in the course of connective tissue disease: report of 34 cases. J Rheumatol. 1994;21:246-51.

63. Ognibene FP, Shelhamer JH, Hoffman GS, et al. Pneumocystis carinii pneumonia: a major complication of immunosuppressive therapy in patients with Wegener's granulomatosis. Am J Respir Crit Care Med. 1995;151:795-9.

64. Limper AH, Knox KS, Sarosi GA, et al. An official American Thoracic Society statement: Treatment of fungal infections in adult pulmonary and critical care patients. Am J Respir Crit Care Med. 2011;183:96-128.

65. Hugle B, Solomon M, Harvey E, et al. Pneumocystis jiroveci pneumonia following rituximab treatment in Wegener's granulomatosis. Arthritis Care Res (Hoboken). 2010;62:1661-4.

66. Kronbichler A, Kerschbaum J, Gopaluni S, et al. Trimethoprim-sulfamethoxazole prophylaxis prevents severe/life-threatening infections following rituximab in antineutrophil cytoplasm antibody-associated vasculitis. Ann Rheum Dis. 2018;77:1440-7.

67. Monach PA, Arnold LM, Merkel PA. Incidence and prevention of bladder toxicity from cyclophosphamide in the treatment of rheumatic diseases: a data-driven review. Arthritis Rheum. 2010;62:9-21.

68. West NJ. Prevention and treatment of hemorrhagic cystitis. Pharmacotherapy. 1997;17:696-706.

69. Takamoto S, Sakura N, Yashiki M, Kojima T. Inactivation of acrolein by sodium 2-mercaptoethanesulfonate using headspace-solid-phase microextraction gas chromatography and mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2003;791:365-9.

70. HJ BM, RHWM D. Toepassing MESNA bij Cyclofosfamide voor de behandeling van autoimmuunziekten. Nederlandse Vereniging voor Reumatologie. 2012.

71. Klemmer PJ, Chalermskulrat W, Reif MS, Hogan SL, Henke DC, Falk RJ. Plasmapheresis therapy for diffuse alveolar hemorrhage in patients with small-vessel vasculitis. Am J Kidney Dis. 2003;42:1149-53.

CASE REPORT

A rare case of Waldenström's macroglobulinaemia-associated cryoglobulinaemia vasculitis

M.M.J. Burgers[1#*], J.A.A. Meijer[1], E.J.H.M. van de Weijgert[1], E. de Jongh[1]

[1]Departement of Internal Medicine, Albert Schweitzer Hospital, Dordrecht, the Netherlands.
*Corresponding author: mmjburgers@gmail.com;
[#]current: Erasmus Medical Centre, Rotterdam, the Netherlands

ABSTRACT

This case report presents a patient with vasculitis as a presenting symptom of type I cryoglobulinaemia due to lymphoproliferative disease. This is an uncommon cause of vasculitis, but important to recognise, as it influences treatment decisions. We discuss the differential diagnosis and extensive diagnostic approach of vasculitis. Above all, this case emphasizes that even a limited quantity of paraproteins can cause severe symptoms.

KEYWORDS

Cryoglobulinaemia, paraproteinemia, vasculitis, Waldenström's macroglobulinaemia

What was known on this topic?
Vasculitis is a condition with a varied and nonspecific clinical presentation and extensive differential diagnosis. Clinical presentation depends on affected organs. The combination of constitutional symptoms and (multiple) organ dysfunction raises the suspicion of vasculitis. Cryoglobulinaemia is a phenomenon of precipitation of immunoglobulins with cold temperature. Monoclonal cryoglobulins are strongly associated with underlying haematological disease. Typically, clinical presentation is related to vaso-occlusion and occur in cold body parts. Rarely, small vessel vasculitis is described in these patients. Diagnostic approach and treatment aim to target the underlying condition.

What does this add?
This case highlights that even a limited quantity of paraproteins can cause severe symptoms and require treatment. Furthermore, type I cryoglobulinaemia due to lymphoproliferative disease is an uncommon cause of vasculitis. We describe how the extensive diagnostic process of a suspected vasculitis can result in detection of a rare underlying cause with consequences for treatment.

INTRODUCTION

Vasculitis has a varied and nonspecific clinical presentation and extensive differential diagnosis.[1,2] In this case report, we describe the diagnostic process from suspected vasculitis to established small vessel vasculitis based on monoclonal cryoglobulinaemia due to an underlying haematological disease. This case shows an uncommon cause of vasculitis and highlights that even a limited quantity of paraproteins can cause severe morbidity.

CASE REPORT

A 78-year-old man was admitted to our hospital with malaise, night sweats, and weight loss. Six months before, he was evaluated by a neurologist because of axonal polyneuropathy. Work-up revealed an IgM-kappa paraproteinaemia of < 1 g/l. He did not suffer from B symptoms at that time and there were no signs of lymphadenopathy or bone marrow invasion. IgM antibodies against myelin-associated glycoprotein were not detected. His further medical history reported a coronary artery bypass graft and treated tuberculosis in childhood.

For the last two weeks, he reported malaise, night sweats, and weight loss. He felt progressive pain and stiffness in his legs during exercise, which was different from the previous neuropathic pain. Furthermore, he noticed a right-sided foot drop.
On physical examination there was no lymphadenopathy and examination of heart, lungs, and abdomen was unremarkable. We noticed livedo reticularis on both legs and bilateral pitting oedema. There were no signs of acrocyanosis or purpura. Peripheral pulsations were intact. Neurologic examination showed a paresis of the dorsiflexors of the right foot, suggestive for peroneus nerve damage. Initial laboratory tests showed mild normocytic anaemia, mild elevated C-reactive protein, and elevated creatinine kinase with normal liver and kidney function.

Because of the constitutional symptoms, livedo reticularis, and suspected myositis and mononeuritis multiplex, we considered a small vessel vasculitis (table 1). In order to identify the type of vasculitis and to consider the differential diagnosis of a malignancy or infection, additional laboratory tests (table 2) and a positron emission tomography/computed tomography (PET-CT) scan followed.[3,4] The PET-CT scan showed elevated uptake in the right upper leg and an additional magnetic resonance imaging (MRI) scan was suggestive for inflammatory myopathy of the vastus lateralis muscle. Biopsy of skin, muscle, and fascia from the right quadricep muscle showed perivascular infiltrates of predominantly lymphocytes with destruction of the small vessel walls. Together with fibrinoid degeneration, signs of leucocytoclasia, and thrombosis of the lumen, a leucocytoclastic vasculitis was diagnosed. Additional laboratory tests showed no evidence for an auto-immune aetiology or systemic rheumatic disease. Cryoglobulinaemic vasculitis was diagnosed due to the presence of cryoglobulins, which could be typed as type I (monoclonal IgM kappa). Bone marrow biopsy revealed

Table 1. *Symptoms related to vasculitis*

Constitutional symptoms
Fever of unknown origin, fatigue, weight loss, decreased appetite, and/or night sweats
Signs of ischaemia
Multiple affected organs
Eyes: uveitis, optic neuritis
Skin: erythema nodosum, (sub)cutaneous nodules, ulcers, palpable purpura
Ears, nose, and throat: jaw claudication, sinusitis, chronic otitis media, polyposis
Lungs: parenchymal nodules, haemorrhage
Heart: aortic regurgitation, peri-/myocarditis, coronary artery vasculitis
Gastrointestinal: abdominal pain, diarrhoea, bleeding, symptoms related to ischaemia
Kidney: glomerulonephritis, symptoms related to stenosis of the renal artery
Peripheral nervous system: mononeuritis multiplex, polyneuropathy
Central nervous system: headache, cerebral vasculitis, stroke

This list is a summary of possible symptoms, for the complete list, see reference 8

Table 2. *Laboratory findings*

	Laboratory findings	Reference values
Haemoglobin (mmol/l)	7.0	8.5-10
MCV (fL)	89	80-100
Leukocytes (10^9/l)	7.7	4-10
Thrombocytes (10^9/l)	216	150-400
Erythrocyte sedimentation rate (mm/1st h)	13	< 10
C-reactive protein (mmol/l)	70	< 3
Creatine kinase (U/l)	2534	< 200
Total IgM (g/l)	1.79	0.45-2.30
M-protein (g/l)	IgM-kappa: < 1	Not detectable
Free light chain ratio	5.47	0.26-1.65
Antinuclear antibodies	Negative	Negative
Anti-MPO antibodies (U/l)	Negative	Negative
Anti-PR3-antibodies (U/l)	< 5	< 5
Lupus anticoagulance	Negative	Negative
Cryoglobulin (serum)	IgM-kappa cryoglo-bulinaemia	Not detectable
Rheumatic factor (kIU/l)	< 10	< 10
Anti-CCP antibodies (U/l)	< 25	< 25
C3 (g/l)	0.69	0.90-1.80
C4 (g/l)	< 0.08	0.16-0.42
Serology for HIV, hepatitis C and B	Negative	Negative

The above, as well as total eosinophils, creatinine level, liver enzymes, and urinalysis are recommended if vasculitis is considered.[2,8]
C3 = complement 3; C4 = complement 4; CCP = cyclic citrullinated peptide; IgM = immunoglobulin M; MCV = mean corpuscular volume; MPO = myeloperoxidase; PR3 = proteinase 3.

slight infiltration of small B-cell non-Hodgkin lymphoma with plasmacytoid differentiation.

Once the diagnosis of symptomatic Waldenström's macroglobulinaemia was confirmed, we started first-line therapy:[5-7] six courses of dexamethasone, rituximab (an anti-CD20 monoclonal antibody), and cyclophosphamide (chemotherapy). After six treatment courses, paraproteins and cryoglobulins were not detectable anymore and creatine kinases – as a marker of myositis – was normalised. His symptoms improved and he went for clinical revalidation to regain condition.

DISCUSSION

Vasculitis

Constitutional symptoms and suspected myositis and mononeuritis multiplex were the main symptoms leading to small vessel vasculitis in this case. When vasculitis is considered, a detailed patient history and careful physical examination is essential, with regard to possible manifestations. Initial laboratory testing should include all tests presented in table 2, as well as eosinophil count, creatinine level, liver enzyme levels, and urinalysis.[1,8] Depending on affected organs, specific tests such as electromyography or liquor tests can be performed. Vascular imaging through ultrasound, PET, CT, or MRI can support the diagnosis of large and medium vessel vasculitis.[9] For small vessel vasculitis, histological examination is necessary to confirm diagnosis and can be best performed in an affected organ.[8] Neutrophilic infiltration surrounding and involving blood vessel walls and fibrinoid necrosis are typical. Leucocytoclasia, extravasation of erythrocytes, necrosis, and thrombi can also be seen. The performed serum tests (auto-immune serology, complement factors, eosinophils) are necessary to differentiate in specific aetiology of vasculitis (table 3). However, a normal biopsy does not exclude vasculitis, as the inflammation can be segmented.

Table 3. *Differential diagnosis of small vessel vasculitis*[1,3,4]

ANCA-associated small vessel vasculitis
Granulomatosis with polyangiitis
Eosinophilic granulomatosis with polyangiitis
Microscopic polyangiitis
Immune complex-mediated vessel vasculitis
IgA vasculitis
Cryoglobulinaemic vasculitis
Monoclonal cryoglobulinaemia, associated with lympho-proliferative disease
Mixed cryoglobulinaemia, associated with infectious and auto-immune disease
Cutaneous leucocytoclastic vasculitis
Hypocomplementemic urticarial vasculitis (anti-C1q antibodies)
Antibody-mediated small vessel vasculitis
Anti-glomerular basement membrane disease

ANCA = anti-neutrophil cytoplasmic antibodies;
IgA = immunoglobulin A

Cryoglobulinaemia

Cryoglobulinaemia is defined as the precipitation of immunoglobulins with cold temperature. Symptomatic cryoglobulinaemia is considered a rare disease in Europe,[10] although exact prevalence is unknown due to the heterogeneity in cause, clinical presentation, and geographical distribution.[11] After immunofixation, three subtypes can be identified.[12] Solely monoclonal immunoglobulins (paraproteins) are referred to as type I; type II comprises both monoclonal and polyclonal immunoglobulins, and type III only polyclonal immunoglobulins. Due to overlapping clinical presentation and pathophysiology, type II and III are also referred to as mixed cryoglobulinaemia; 5-25% of cryoglobulins are monoclonal.[13,14]

The cryoprecipitability of monoclonal immunoglobulins depends on intrinsic factors such as pH, temperature, and absence of sialic acid moieties.[1] The aggregated cryoglobulins seem to saturate the mononuclear phagocyte system activity, possibly by influencing Fc receptor function, resulting in accumulation and deposition of the cryoglobulins in specific organs.[15] Clinical presentation of cryoglobulinaemia is divergent, and in most cases even asymptomatic.[16,17] Symptoms are often secondary to hyperviscosity and vascular occlusion, such as Raynaud phenomenon, acrocyanosis, purpura, cold urticaria, ulcers, or skin necrosis.[1] Leucocytoclastic vasculitis due to type I cryoglobulinaemia is rarely described. A French nationwide study identified 64 patients between 1995 and 2010; before that, only case reports with few patients were published. None of these patients reported typical vaso-occlusive symptoms.[14]

In contrast, leucocytoclastic vasculitis is typically seen in mixed cryoglobulinaemia.[1] Immunoglobulins associate with rheumatoid factor (in contrast to monoclonal cryoglobulins), form immunocomplexes, and precipitate in small and medium-sized vessels. This leads through inflammation and complement activation to leucocytoclastic vasculitis. Mixed cryoglobulinaemia is related to chronic viral infections (particularly hepatitis

C, but also associated with hepatitis B and HIV), autoimmune diseases, and lymphoproliferative disorders.[18] Cryoglobulins could be detected in more than 50% of patients who were infected with chronic hepatitis C virus or HIV.[19]

The diagnostic approach of cryoglobulinaemia is focused on identifying involved organs and underlying disease, as this influences treatment decisions.[1] Renal and/or skin involvement can be diagnosed with urinary analysis, renal, or skin biopsy.[2,8] The type of cryoglobulinaemia has an important role in identifying underlying disease, and therefore quantity and type of immunoglobin and presence of rheumatoid factor should be determined. Type I cryoglobulinaemia is strongly associated with underlying lymphoproliferative disorder, such as Waldenström's macroglobulinaemia or chronic lymphoid leukaemia.[13] Therefore, bone marrow examination is indicated.

Level of paraprotein and severity of symptoms

As our case demonstrates, even a limited quantity of paraprotein can cause cryoglobulinaemia with severe symptoms. A recent retrospective study among 64 patients with type I cryoglobulinaemia could not identify a correlation between paraprotein level and clinical severity.[20] Previous studies already demonstrated that the concentration of paraprotein has little or no prognostic value.[1,21] Therefore, *starting* treatment against Waldenström's macroglobulinaemia is based on symptoms, regardless of the paraprotein level. In the majority of patients with type I cryoglobulinaemia, treatment of underlying disease leads to improvement or stabilisation of symptoms.[13]

CONCLUSION

Vasculitis can be a presenting symptom of type I cryoglobulinaemia due to lymphoproliferative disease, although rarely seen. Vasculitis should be considered in patients with constitutional symptoms and symptoms of multiple affected organs. An extensive diagnostic process is necessary to identify underlying cause. This case emphasises that even very limited quantity of paraproteins can lead to cryoglobulinaemia with severe symptoms.

DISCLOSURES

All authors declare no conflicts of interest. No funding or financial support was received.

REFERENCES

1. Kolopp-Sarda MN, Miossec P. Cryoglobulins: An update on detection, mechanisms and clinical contribution. Autoimmun Rev. 2018;17:457-64.
2. Sharma AM, Singh S, Lewis JE. Diagnostic approach in patients with suspected vasculitis. Tech Vasc Interv Radiol. 2014;17:226-33.
3. Davies DJ. Small vessel vasculitis. Cardiovasc Pathol. 2005;14:335-46.
4. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum. 2013;65:1-11.
5. Buske C, Hoster E, Dreyling M, et al. The addition of rituximab to front-line therapy with CHOP (R-CHOP) results in a higher response rate and longer time to treatment failure in patients with lymphoplasmacytic lymphoma: results of a randomized trial of the German Low-Grade Lymphoma Study Group (GLSG). Leukemia. 2009;23:153-61.
6. Dimopoulos MA, Anagnostopoulos A, Kyrtsonis MC, et al. Primary treatment of Waldenstrom macroglobulinemia with dexamethasone, rituximab, and cyclophosphamide. J Clin Oncol. 2007;25:3344-9.
7. Ioakimidis L, Patterson CJ, Hunter ZR, et al. Comparative outcomes following CP-R, CVP-R, and CHOP-R in Waldenstrom's macroglobulinemia. Clin Lymphoma Myeloma. 2009;9:62-6.
8. Thio H, Voskuyl A, Everdingen J, et al. Richtlijn diagnostiek kleine vaten vasculitis. Utrecht: 2010.
9. Schmidt WA, Blockmans D. Investigations in systemic vasculitis - The role of imaging. Best Pract Res Clin Rheumatol. 2018;32:63-82.
10. Rath A, Kelly D. Prevalence of mixed cryoglobulinaemia syndrome and circulating cruoglobulins in a population-based survey: the Origgio study. Orphanet Report Series, Rare Diseases collection [Internet]. 2019.
11. Monti G, Saccardo F, Castelnovo L, et al. Prevalence of mixed cryoglobulinaemia syndrome and circulating cryoglobulins in a population-based survey: the Origgio study. Autoimmun Rev. 2014;13:609-14.
12. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. Am J Med. 1974;57:775-88.
13. Sidana S, Rajkumar SV, Dispenzieri A, et al. Clinical presentation and outcomes of patients with type 1 monoclonal cryoglobulinemia. Am J Hematol. 2017;92:668-73.
14. Terrier B, Karras A, Kahn JE, et al. The spectrum of type I cryoglobulinemia vasculitis: new insights based on 64 cases. Medicine (Baltimore). 2013;92:61-8.
15. Gorevic PD. Rheumatoid factor, complement, and mixed cryoglobulinemia. Clin Dev Immunol. 2012;2012:439018.
16. Neel A, Perrin F, Decaux O, et al. Long-term outcome of monoclonal (type 1) cryoglobulinemia. Am J Hematol. 2014;89:156-61.
17. Vos J, Minnema M, Wijermans P, et al. Richtlijn voor diagnostiek en behandeling van de ziekte van Waldenström. Ned Tijdschr Hematol. 2012;9:219-31.
18. Cacoub P, Comarmond C, Domont F, Savey L, Saadoun D. Cryoglobulinemia Vasculitis. Am J Med. 2015;128:950-5.
19. Ramos-Casals M, Munoz S, Medina F, et al. Systemic autoimmune diseases in patients with hepatitis C virus infection: characterization of 1020 cases (The HISPAMEC Registry). J Rheumatol. 2009;36:1442-8.
20. Harel S, Mohr M, Jahn I, et al. Clinico-biological characteristics and treatment of type I monoclonal cryoglobulinaemia: a study of 64 cases. Br J Haematol. 2015;168:671-8.
21. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol. 2003;30:110-5.

CASE REPORT

Acute kidney failure after intra-articular use of gentamicin sponge

E.P.A.T. Gommans[1,2]*, A.L.H.J. Aarnoudse[3], R.J.A. van Wensen[4], A.A.W. van Erp-van Boekel[5], R.J.E. Grouls[6], C.M.J. van der Linden[1]

[1]Department of Geriatrics, Catharina Hospital, Eindhoven, the Netherlands; [2]Current position: Geriatics, St Anna Hospital Geldrop, the Netherlands; Departments of [3]Internal medicine, [4]Orthopedics, Catharina Hospital, Eindhoven, the Netherlands; [5]Netherlands Farmacovigilance Centre Lareb, the Netherlands; [6]Department of Clinical Pharmacy, Catharina Hospital, Eindhoven, the Netherlands.
*Corresponding author: e.gommans@st-anna.nl

SUMMARY

An 83-year-old man developed acute kidney failure after intra-articular use of gentamicin sponges for a periprosthetic hip infection. Haemodialysis was necessary for clearance of gentamicin, and for kidney function replacement. It is important to be aware that there is a risk of renal toxicity due to gentamicin when using a locally applied sponge.

KEY WORDS

Aminoglycoside, gentamicin, nephrotoxicity

What was known on this topic?
The aminoglycoside gentamicin has a potent antibacterial effect but also a high risk of toxicity. The incidence of nephrotoxicity is estimated to be 10-25%.[1]

What does this add?
Intra-articular use of gentamicin can cause toxic plasma concentrations and acute kidney failure.

INTRODUCTION

In systemic application of aminoglycosides, therapeutic drug monitoring (TDM) is necessary and considered usual care. This helps to achieve optimal dosage and intervals in order to achieve maximum efficacy and safety. High trough levels increase the risk of nephrotoxicity.[2] TDM after the implantation of local gentamicin devices, such as beads and sponges, is not part of usual care. We describe an 83-year-old patient with toxic gentamicin concentrations, complicated by severe renal insufficiency. We also formulate an advice on how to prevent this in other patients.

CASE REPORT

An 83-year-old man was admitted with signs of acute periprosthetic joint infection. His medical history included hypertension and atrial fibrillation, and he used metoprolol 100 mg, chlortalidone 25 mg, quinapril 10 mg, and acenocoumarol. Symptoms were wound leakage and malaise. Leucocyte count was 11*10^9/l and C-reactive protein (CRP) was 57 mg/l. Treatment with flucloxacillin was started intravenously, and debridement of the hip with intra-articular placement of two 130 mg gentamicin sponges. In the four days following, the patient suffered from persistent malaise, vomiting, and mild hypotension (RR 100/50 mmHg). Antihypertensive medication was interrupted and intravenous fluid therapy was started. Despite this, the patient was progressively oliguric. Four days after the operation, laboratory tests revealed a gentamicin level of 2.6 mg/l, creatinine of 542 µmol/l, and estimated glomerular filtration rate (eGFR) of 8 ml/min. A previous creatinine value of 84 µmol/l was measured three months earlier. The patient had normal sodium and potassium values, calcium 1.91 mmol/l, and phosphate 2.28 mmol/l. No eosinophilia was found. Urinary analysis showed no proteinuria, only low leucocyte and erythrocyte counts, and no casts. Target value for gentamicin trough

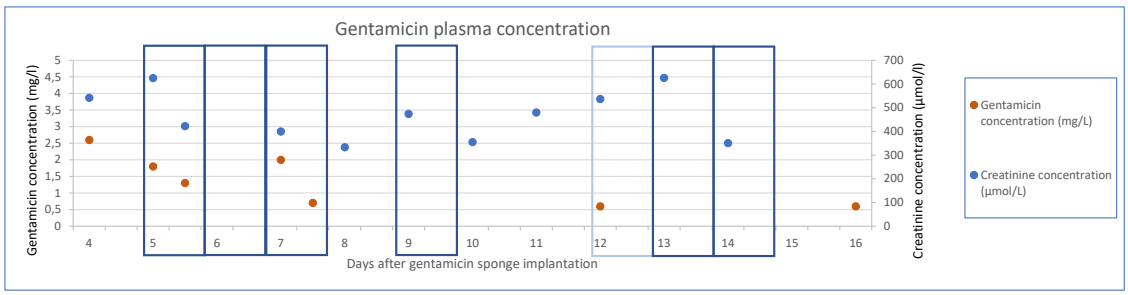
levels in our hospital is < 0.5 mg/l; in literature, a target value < 1.0 mg/l is also mentioned.[2] We suspected acute tubular necrosis, on the one hand caused by gentamicin toxicity, and on the other hand by the moderately low blood pressure, which was related to infection and dehydration, and use of a thiazide and angiotensin converting enzyme (ACE)-inhibitor. Tubulo-interstitial nephritis caused by flucloxacillin was considered less likely, because of the very recent start of flucloxacillin and absence of typical abnormalities in urinary analysis. Post-renal obstruction was ruled out by ultrasound. Haemodialysis was started as renal function replacement therapy and to lower the gentamicin concentration in order to reduce further toxicity. Removal of the sponge was not possible due to degradation of these sponges within a few days. Gentamicin level decreased to 1.8 mg/l after starting intravenous fluid therapy and to 1.3 mg/l after the first dialysis session. Two days afterwards, plasma level had increased to 2.0 mg/l again while the fluid status of the patient had not changed. Dialysis was necessary on three consecutive days before gentamicin levels had decreased to a level of 0.7 mg/l. Sixteen days and six dialysis sessions after implantation of the sponge, the plasma concentration was still 0.6 mg/l. After five weeks, eGFR had recovered to 18 ml/min and dialysis was ended. After that, eGFR further recovered up to 50 ml/min.

DISCUSSION

With gentamicin sponges, high concentrations of gentamicin are achieved locally during approximately three days.[3] The absorption of gentamicin in systemic circulation varies, but a study by Swieringa et al. demonstrates that concentrations in plasma generally drop below 2.0 mg/l within 24 hours and decrease after that.[4] In the case we describe, the release profile of the sponge seems to deviate from the aforementioned profile. Extended release is suspected, based on the course of plasma concentrations, which were repeatedly increasing or at the same level as before the previous dialysis session (figure 1). Redistribution after dialysis must be considered, since Sowinski et al. shows a median gentamicin redistribution percentage of 38.7% after dialysis.[5] Redistribution however, does not sufficiently explain the course of plasma levels in this patient, who exhibited levels still above 0.5 mg/l up to day 16.

The incidence of nephrotoxicity due to intravenously administered gentamicin is 10% to 25%.[1] Accumulation of gentamicin in the tubulus cells leading to acute tubular necrosis is the most important mechanism of toxicity. This risk is increased in patients with nephrotoxic co-medication and hypotension. This case report demonstrates that locally applied gentamicin sponges may also cause toxic plasma levels and renal failure. The Netherlands Pharmacovigilance Centre Lareb reported one other case concerning toxic gentamicin levels and new renal insufficiency after the insertion of gentamicin sponges in a 73-year-old woman with an infected hip prosthesis. That patient needed to be treated with continuous veno-venous haemofiltration to limit loss of kidney function.[6,7] Lareb also received four more notifications about patients with an increased creatinine blood level after insertion of gentamicin sponges to treat an infected hip replacement. Swieringa et al. describe a rise in serum creatinine in 10 out of 12 patients treated with gentamicin sponges, when using 4 to 6 sponges per patient.[8]

Figure 1. *Gentamicin plasma concentrations and creatinine concentrations per day. Dialysis sessions are indicated with the boxes. (On day 12, dialysis was started but interrupted because of problems with the dialysis catheter)*



In order to treat gentamicin toxicity, it is important to discontinue gentamicin treatment, start supportive care by fluid administration, and to discontinue other nephrotoxic agents. Dialysis can be indicated for clearance of the causative agent, especially when using gentamicin sponges, since removal of the sponge is not possible. Acute tubular necrosis caused by gentamicin is often reversible, as was the case in our patient. Ototoxicity caused by gentamicin is often not reversible. In our patient, no objective tests of hearing and vestibular function were done since there were no subjective symptoms of ototoxicity.

After this case of intoxication, the monitoring of patients receiving gentamicin sponges and beads improved in our hospital: physicians must prescribe these materials via the electronic medication prescribing system. This enables extra monitoring by the pharmacist, who may suggest follow-up of creatinine and gentamicin levels, and in certain cases, may advise consultation by a nephrologist. Furthermore, this case led to increased awareness amongst physicians that measurement of eGFR is necessary before using local gentamicin sponges.

CONCLUSION

The use of gentamicin sponges may cause systemic toxicity. The release profile to the bloodstream of a gentamicin sponge is variable. Extended release of the sponge increases the risk of nephrotoxicity.

Determination of eGFR is essential before deciding to apply a gentamicin sponge. If a sponge is implanted despite impaired renal function, extra attention to optimal fluid status and other nephrotoxic agents is necessary, as well as follow-up of eGFR and gentamicin levels after the procedure. Gentamicin sponges should be registered in the electronic prescribing system in order to facilitate extra monitoring.

REFERENCES

1. Lopez-Novoa JM, Quiros Y, Vicente L, Morales A, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int. 2011;79:33-45.
2. S. Coenradie, D.J. Touw. TDM-monografie gentamicine [Internet]. 2005 [date accessed 20 November 2018]. Available from: https://tdm-monografie.org/monografie/gentamicine.
3. Ruszczak Z, Friess W. Collagen as a carrier for on-site delivery of antibacterial drugs. Advanced drug delivery reviews 55. 2003:1679-98.
4. Swieringa A, Goossen J, Jansman F, Tulp N. In vivo pharmacokinetics of a gentamicin-loaded collagen sponge in acute periprosthetic infection: serum values in 19 patients. Acta Orthop. 2008;79:637-42.
5. Sowinski K, Magner S, Lucksiri A, Scott M, Hamburger R, Mueller B. Influence of hemodialysis on gentamicin pharmacokinetics, removal during hemodialysis, and recommended dosing. Clin J Am Soc Nephrol. 2008;3:355-61.
6. Verweij S, Schut N, Zwartelé E, Becker M. Nierfunctieverlies door gentamicine-implantatiesponzen. Ned Tijdschr Geneeskd. 2018;162:D2077.
7. Netherlands pharmacovigilance centre Lareb. Database accessed 05 December 2018.
8. Swieringa A, Tulp N. Toxic serum gentamicin levels after the use of gentamicin-loaded sponges in infected total hip arthroplasty. Acta Orthopaedica. 2005;76:75-7.

PHOTO QUIZ

Raccoon sign

M. van der Huizen*, E. Jacobs

Department of Internal Medicine, Elkerliek Hospital, Helmond, the Netherlands.
*Corresponding author: m.vanderhuizen@gmail.com

Figure 1. *Pigment spots around the eyes*

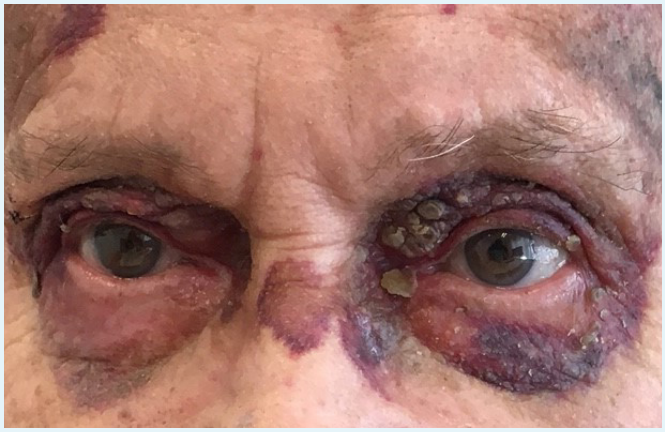
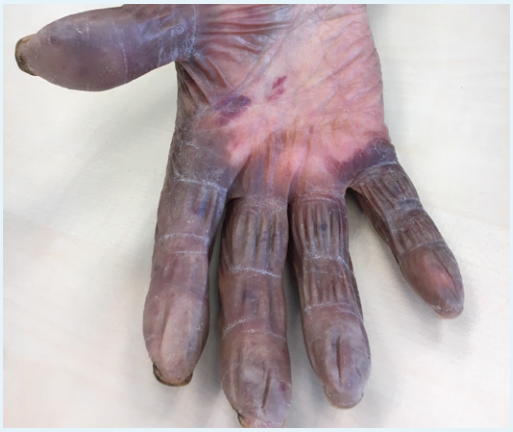


Figure 2. *Blue and purple spots on the hands*



CASE REPORT

An 80-year-old man presented to our outpatient clinic with purple and blue pigment spots on his fingers, hands, and face, particularly around his eyes. He was diagnosed with a smouldering IgG lambda multiple myeloma six years earlier, for which he was receiving follow-up care. Laboratory results showed IgG lambda values of 30 g/l, an isolated anaemia (haemoglobin 7.4 mmol/l) and normal coagulation values. Bone marrow biopsy and aspiration were repeated and demonstrated an increase in plasma cells.

WHAT IS THE DIAGNOSIS?

See page 91 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 90)

RACCOON SIGN

DIAGNOSIS

The patient was diagnosed with amyloid light-chain (AL) amyloidosis.[1,2] Originally, he presented with a smouldering multiple myeloma. During follow up care, he developed purpura on his hands. A rectal biopsy was performed suspecting amyloidosis, but this was negative for amyloid depositions. The purpura where only present when the patient used gardening tools and resolved spontaneously.

Over the following years, the purpura worsened. A lip biopsy was again negative for amyloid. After this, a skin biopsy of the affected area on his hand was performed. This was positive for Congo red staining and demonstrated a typical apple-green birefringence in polarised light. Furthermore, it was positive for amyloid P and lambda staining and negative for amyloid A and kappa staining. The diagnosis AL amyloidosis was made based on these biopsy results in combination with his known monoclonal plasma cell disorder in the bone marrow.[3] The bone marrow biopsy was not revised for amyloid deposition. The serum level of lambda measured was 722 mg/l, with a kappa/lambda ratio of 0.02.

Our patient was treated with various lines of therapy. During treatments, the purpura seemed to remain steady, but worsened as the disease became refractory. Eventually our patient also developed periorbital distribution of purpura, the highly characteristic raccoon sign.

AL amyloidosis is caused by deposition of protein derived from immunoglobulin light chain fragments associated with monoclonal plasma cell disorders. The rare systemic disease can present with various symptoms including proteinuria, hepatosplenomegaly, and cardiomyopathy. The diagnosis can be made based on serum and urine protein electrophoresis with immunofixation, serum free light chain ratio analysis, in combination with an abdominal fat pad biopsy and bone marrow biopsy.[4] In our case, a rectal biopsy was initially performed, which has a slightly lower sensitivity (50-70%) compared to an abdominal fat pad biopsy (60-80%), but a higher sensitivity than a bone marrow biopsy (50-55%) and skin biopsy (50%). Skin involvement of amyloidosis is seen in approximately 40% of patients with AL amyloidosis. It is caused by amyloid depositions and clinical presentation depends on the site of deposition. Presentations include waxy thickening, easy bruising, and subcutaneous nodules or plaques. The purpura in our patient are probably caused by small vessel amyloid deposition in combination with minor trauma. Amyloidosis is also associated with bleeding diathesis such as factor X deficiency.[5] Factor X and other coagulation tests were normal in our patient. Our patient never showed signs of other organ involvement.

With this case, we hope to establish more knowledge about the skin manifestations of amyloidosis, its manifestation in the course of an apparently normal multiple myeloma disease, and to demonstrate the difficulty in diagnosing this rare disease.

REFERENCES

1. Inokuchi R, Tagami S, Maehara H. An elderly woman with bilateral raccoon eyes. Emerg Med J. 2016;33:781.
2. Varim C, Ergenc H, Uyanik MS, et al. A very rare presentation of Multiple Myeloma: unilateral Raccoon eye. Open Access Maced J Med Sci. 2015;3:436-8.
3. Kumar S, Sengupta RS, Kakkar N, Sharma A, Singh S, Varma S. Skin involvement in primary systemic amyloidosis. Mediterr J Hematol Infect Dis. 2013;5:e2013005.
4. Gertz MA. Immunoglobulin light chain amyloidosis diagnosis and treatment algorithm 2018. Blood Cancer J. 2018;8:44.
5. Mumford AD, O'Donnell J, Gillmore JD, Manning RA, Hawkins PN, Laffan M. Bleeding symptoms and coagulation abnormalities in 337 patients with AL-amyloidosis. Br J Haematol. 2000;110:454-60.

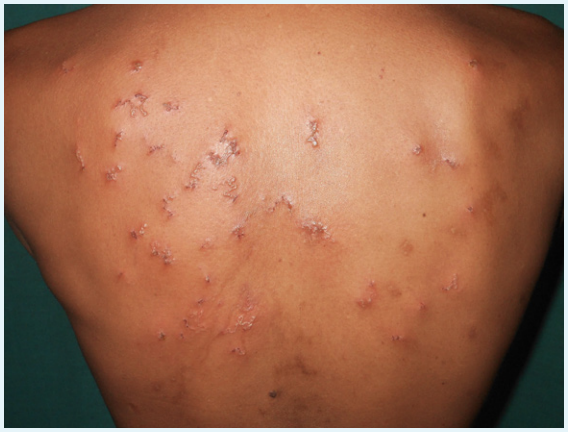
PHOTO QUIZ

Multiple curvilinear lesions on a patient's back

A. Sil*, G.S. Mukherjee, D.B. Bhanja, A. Panigrahi

Department of Dermatology, Venereology, and Leprosy, R.G. Kar Medical College, Kolkata, West Bengal, India. *Corresponding author: abheek.sil@gmail.com

Figure 1. *Multiple serpentine skin-coloured to erythematous tracts distributed over the entire back*



CASE REPORT

An otherwise healthy 39-year-old Indian labourer presented with five-day history of intensely pruritic, progressive, serpiginous skin eruptions on his back. A few days prior to onset of symptoms, he had worked at a construction site carrying sandbags over his bare back. He had no other complaints suggestive of any systemic involvement. Cutaneous examination revealed multiple skin-coloured to erythematous thread-like curvilinear tracts of various sizes (3 to 14 cm in length) distributed haphazardly over the entire back (figure 1); few tracts showing healing at one end and progression at the other were also noted. Laboratory examination was notable for mild anaemia and eosinophilia (absolute eosinophil count 1150 cells/µl).

WHAT IS YOUR DIAGNOSIS?

See page 93 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 92)

MULTIPLE CURVILINEAR LESIONS ON A PATIENT'S BACK

DIAGNOSIS

Based on history and characteristic cutaneous features, a diagnosis of multifocal cutaneous larva migrans (CLM) was established. Oral ivermectin (12 mg; 200 µg/kg) prescribed on two successive days, led to symptomatic relief and cessation of tract extension.

CLM, also termed as "creeping eruption", is a parasitic infestation produced by burrowing of the larva of *Ancylostoma braziliense*.[1] It is most commonly found in tropical and sub-tropical geographic areas, and is endemic in the Caribbean, Central and South America, Africa, and South-East Asia. The larva enters intact or abraded skin following exposure to soil contaminated with faeces.[2] Feet, hands, buttocks, and genitalia are usually affected. Secondary infection and rarely Loeffler syndrome (allergic pulmonary response) may complicate the creeping eruption. Oral ivermectin or albendazole provides excellent response.[3]

The purpose of documenting this condition is to highlight the unusual features of this common tropical condition - mode of acquisition, involvement of the entire back, and multifocal presentation. In this era of global migration, clinicians worldwide should promptly diagnose and treat such endemic disorders.

REFERENCES

1. Karthikeyan K, Thappa D. Cutaneous larva migrans. Indian J Dermatol Venereol Leprol. 2002;68:252-8.
2. Paul Y, Singh J. Cutaneous Larva Migrans in an Infant. Indian Pediatr. 1994;31:1089-91.
3. Cayce KA, Scott CM, Phillips CM, Frederick C, Park HK. What is your diagnosis? Cutaneous larva migrans. Cutis. 2007;79:429, 435-6.

LETTER

The book of *Genesis* and physician-patient communication

A. Schattner

Faculty of Medicine, Hebrew University and Hadassah Medical School, Jerusalem
Corresponding author: amischatt@gmail.com

Dear Editor,

Much can be learned from the book of *Genesis*. Surprisingly, a profound piece of advice applicable to all patient encounters can be found hidden between the lines. After being sold by his brothers, Joseph, son of Jacob, is brought to Egypt and finds himself in prison together with two of King Pharaoh's noblemen (*Genesis*, 40). One morning, he notices that they seem different and sour [*Observation*]. They do not say anything, and Joseph goes on to ask them if anything is the matter [*Curiosity*, actively pursued; *Initiative*]. Upon hearing that they both had disturbing dreams, Joseph encourages them to narrate their dreams, suggesting that he may be able to help [*Interest*, *Commitment*], and then ably interprets their dreams for them [*Support*]. When later, Pharaoh himself has a hard-to-decipher recurring dream, the nobleman who was reinstated remembers Joseph and recommends his abilities to the king. Joseph is duly brought before the king where, due to his clear interpretation and bright idea for dealing with the looming crisis revealed by the dreams, he is freed and appointed a prince, second only to the king.

All of this would not have happened had Joseph not demonstrated constant mindfulness, sensitivity to nonverbal clues, curiosity, and other essential qualities (above in square brackets) identified by research as being widespread among exemplary humanistic clinicians.[1-3]

Unfortunately, these qualities are often neglected and missing in most settings;[4,5] a neglect associated with suboptimal care and health outcomes for patients, and with missed information, hampered meaning, and increased burnout for physicians. Conversely, the potential rewards of Joseph's attitude are symbolised by his rise from prison to prince.

With the current time-constrained, information-packed encounters, and patients' common use of subtle cues to try and surface highly significant emotional and contextual issues,[4] the onus is on us, as providers, to observe and notice, be curious, ask, and react, displaying empathy and commitment – as Joseph did according to the text of *Genesis*.

REFERENCES

1. Churchill LR, Schenck D. Healing skills for medical practice. Ann Intern Med. 2008;149:720-4.
2. Chou CM, Kellom K, Shea JA. Attitudes and habits of highly humanistic physicians. Acad Med. 2014;89:1252-8.
3. Schattner A. Curiosity. Are you curious enough to read on? J Royal Soc Med. 2015;108:160-4.
4. Levinson W, Gorawara-Bhat R, Lamb J. A study of patient clues and physician responses in primary care and surgical settings. JAMA. 2000;284:1021-7.
5. Zimmermann C, Del Piccolo L, Finset A. Clues and concerns by patients in medical consultations: a literature review. Psychol Bull. 2007;133:438-63.