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Immunogenicity induced by botulinum toxin injections for limb spasticity: a systematic review

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Abstract

Background. The imputability of neutralizing antibodies (NABs) in secondary non-response (SnR) to botulinum toxin (BoNT) injections for limb spasticity is still debated.

Objective. This systematic literature review aimed to determine the prevalence of NABs after BoNT injections for limb spasticity and analyze their determinants and their causal role in SnR.

Methods. We searched MEDLINE via PubMed, Cochrane and Embase databases for articles published during 1990-2018. Two independent reviewers extracted the data and assessed the quality of studies with a specific scale (according to PRISMA and STROBE guidelines). Because the techniques used to detect NABs did not influence the results, we calculated the global (all studies) sensitivity and specificity of NAB positivity to reveal SnR.

Results. We included 14 articles published from 2002 to 2018 (including an epublication) describing 5 randomized controlled trials and 5 interventional and 4 observational studies. The quality was satisfactory (mean score 18/28 arbitrary units). NAB detection was the primary criterion in 5 studies and a secondary criterion in 9. In total, 1234 serum samples for 1234 participants (91% with stroke) were tested after BoNT injection. NAB prevalence was about 1%, with no significant difference among formulations. NAB positivity seemed favoured by long-duration therapy with high doses and a short interval between injections. The identification of non-response by NAB positivity had poor global sensitivity (56%) but very high specificity (99.6%). No consensual criteria were used to diagnose non-response to BoNT injection.

Conclusions. NAB prevalence is much lower after BoNT treatment for limb spasticity than cervical dystonia. Consensual criteria must be defined to diagnose non-response to BoNT injection. Because immunogenicity is not the most common cause of non-response to BoNT injection, NABs should be sought in individuals with SnR with no other cause explaining the treatment inefficacy. A test with 100% specificity is recommended. In cases for which immunogenicity is the most likely cause of non-response to BoNT injections, some biological arguments suggest trying another BoNT, but no clinical evidence supports this strategy.

Keywords. limb spasticity, botulinum toxin, immunogenicity, resistance to treatment, neutralizing antibody

Introduction

Spasticity is a muscle overactivity resulting from hyperexcitability of the stretch reflex [1]. It may have negative consequences for daily living and often causes limitations of activities, pain and discomfort. Botulinum toxin type A (BoNT-A) injection has become a first-line treatment for spasticity and for many other diseases involving muscle overactivity (blepharospasm, cervical dystonia). The efficacy of BoNT injection for these forms of muscle hyperactivity might have a common course: for some patients, a period of good clinical response is followed by loss of efficacy leading to repeated injections with increasing doses [2], called secondary non-response (SnR) [3].

Different interpretations have been proposed to explain this SnR. The one referring to the immunogenicity of the BoNT has been prominent in the last 2 decades [4–8], which has induced increasing interest in BoNT neutralizing antibodies (NABs). However, this enthusiasm has been tempered by the difficulty in proving a causal link between the existence of NABs and SnR [9–11]. This issue is still debated. Moreover, factors suspected to influence the development of NABs have been insufficiently analyzed for each form of muscle hyperactivity. Despite arguments to support the existence of a relation between SnR and BoNT immunogenicity for cervical dystonia [6, 12, 13], the situation for limb spasticity is less clear. However, spasticity can benefit from high cumulative doses (CDs), mostly with repeated injections, which are 2 factors suggested to contribute to NAB production[14].

Surprisingly, the causality of NABs to BoNT in SnR has never been specifically analyzed for limb spasticity. The objective of this systematic review was to determine the prevalence of NABs in people receiving BoNT injections for limb spasticity and analyze their possible determinants and their causality in SnR.

Methods

Study eligibility

The English literature was systematically reviewed by 2 independent perusals in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (www.prisma-statement.org). We searched MEDLINE via PubMed, Cochrane and Embase databases for articles published from 1990 to 2018 (one paper published in electronic version at the end of 2018 appeared in hard copy in January 2019) by using the keywords "botulinum toxin" AND ("antibody" OR "immunogenicity") AND ("spasticity" OR "limb dystonia"). Eligibility criteria were studies investigating BoNT immunoresistance in a therapeutic strategy for limb spasticity or dystonia of the skeletal muscles in humans. We excluded articles concerning BoNT injections for other symptoms such as cervical dystonia, blepharospasm, facial hemispasm, dysphagia, and overactive bladder. Review papers were read but not analyzed for the present systematic review. References of the included articles were checked for eligible articles.

Quality analysis

A 14-item specific scale to assess study quality was based on the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) principles and was developed by consensus among the 4 authors. Each item was categorized between the introduction, method, results, discussion and conclusion. The items covered domains such as study design, recruitment and description of participants, and global quality (Table 1). The score was expressed in arbitrary units (a.u.) ranging from 0 to 28 (high quality). LM and AD independently evaluated quality, and disagreements were resolved after discussion.

Techniques used to detect NABs

The gold standard to quantify NABs is the *in vitro* quantitative test, namely, the mouse hemidiaphragm assay [13, 27]. A threshold of 0.30 mU/ml is used to detect NABs with 100% specificity[25, 27]. The mouse protection assay has a detection limit of 5 to 10 mU/ml, for sensitivity 5 to 6 times lower than that of the mouse hemidiaphragm assay, but still with 100% specificity [13, 28]. The immunoprecipitation assay, with a detection threshold from 0.30 to 1 mU/ml, seems less sensitive than the mouse hemidiaphragm assay for low-level NABs and is also less specific (80–89%)[28]. Since the techniques used to detect NABs did not influence the results, we calculated the global (all studies) sensitivity and specificity of the identification of SnR by NAB positivity.

Statistical analysis

Data are presented as mean (SD) or number (%).

Results

Included studies

We retrieved 192 articles published up to December 31, 2018 (including one epublication) (Fig. 1). After reading titles and abstracts, we selected 32 articles, but excluded 10. Finally, 12 articles were considered relevant and 2 additional articles were found from references. All 14 articles analyzed were published from 2002 to 2018: 5 described randomized controlled trials [2, 15–18], 5 prospective interventional studies[19–23], and 4 observational studies[11, 24–26]. Only 1, a prospective observational study, was conducted independent of industry support [24].

The mean (SD) quality of the 14 selected articles was 18 (3.7) a.u. (range 15 to 25), for a satisfactory level (Table 2). These 14 studies included a total of 1234 participants, with a relatively large sample size (> 100) in 7 studies (Table 3). Stroke represented the main disease

(91.3% of participants); it was exclusively investigated in 9 studies and investigated with other diseases in 3. Other diseases were cerebral palsy (6% of participants exclusively investigated in 1 study) and less frequently traumatic brain injury, multiple sclerosis, and hereditary spastic paraparesis.

The experimental protocol was always well described. Detection of NABs was the main criterion in 4 studies (133 participants) with different designs [11, 18, 24, 25]. NAB detection was a secondary criterion in the 9 other studies focused on the efficacy (mainly impairment) and safety (adverse events) of BTX injections.

General characteristics of BTX treatment

BTX formulations

Overall, 13 studies used BoNT injections and 1 used BoNT-B injections [15]. Onabotulinum toxin A (Botox®) was used in 3 studies [2, 16, 19], incobotulinum toxin A (Xeomin®) in 3 studies [17, 21, 22] and abobotulinum toxin A (Dysport®) in 1 study, electively [23] (Table 4). Several studies used 2 or 3 products, in different patients or successively in the same patients. abobotulinum toxin A and onabotulinum toxin A were both used in 3 studies [11, 24, 25], and abobotulinum toxin A, onabotulinum toxin A and incobotulinum toxin A were used in 1 study[26]. One study used a new formulation called Meditoxin® (rimabotulinumtoxin B) which is currently available in Korea[20].

Injection sites

Injection sites greatly varied. Six studies focused on the upper limb, particularly elbow, wrist, and finger flexors. Three studies did not specify the injection site(s) [11, 24, 26] and no study analyzed the effect of injection sites on the occurrence of NABs.

Prevalence and occurrence of NABs after BoNT injections

The *in vitro* quantitative test, mouse hemidiaphram assay, was used in 6 studies [11, 17, 21, 22, 24, 26], the mouse protection assay in 6 studies [2, 16, 19, 20, 23, 25], and the immunoprecipitation assay in 3 studies, always in association with another test [17, 20, 21]. The proportion of studies that detected NABs was approximately the same whatever the technique used: 3/5 with the mouse hemidiaphram assay and 4/8 with another technique.

A total of 1234 serum samples were tested. NABs were found in 32 (2.6%) samples, by the mouse hemidiaphram assay in 27 and the mouse protection assay in 5. However, most positive serum samples (17/32) were detected in a single study focused on SnR [11]. When discarding the selected SnR of that study, NABs were detected in only 10/1166 (0.9%) serum samples. Of note, NABs were detected in 5 samples in a single study of 33 patients still responding to treatment[26].

Prevalence and determinants of NAB positivity in cross-sectional studies

For the 5 cross-sectional studies analyzing the presence of NABs as the primary criterion, NAB prevalence was 29/166 (17%). Müller et al. [24] systematically sought NABs in 42 consecutive patients who were repeatedly treated for more than 2 years with abobotulinum toxin A, onabotulinum toxin A or both. Detection was with the mouse hemidiaphragm assay. At the time of NAB detection, participants had received a mean (SD) of 14.2 (6.1) injections, regardless of treatment response. NABs were found in 5 (12%) participants, with some common characteristics: 4 received treatment for longer than 5 years; 4 received high CDs of BoNT (equivalent mouse units [MU-E]: 10923, 8000, 5633 and 5467, respectively, vs mean [SD] 5418 [2.271] MU-E for others); 3 had a treatment interval shorter than 4 months; and 3 were SnR (2 with short treatment intervals). These doses are mean per patients, after a conversion into MU-E, with 1 onabotulinum toxin A unit equivalent to 3 abobotulinum toxin A units. In

this study, patients had received onabotulinum toxin A or abobotulinum toxin A or both sequentially. No difference in prevalence of NABs between the drugs could be found.

Oshima et al. sought NABs by using the mouse protection assay in 38 toxin-naïve children with cerebral palsy receiving treatment for 2 years [18]. The authors compared 2 injection schedules: 1 versus 3 injection sessions per year. Participants received a mean (SD) of 4 (2) injections. NABs were found in 2 participants (5%) who both received 3 injections per year (after 5-6 injections). This finding suggested that the frequency of injections might have been a factor in the development of NABs. No information was given concerning the treatment efficacy in these 2 participants.

Bakheit et al. did not detect any NABs by using the mouse protection assay in 18 participants who had received many injections (mean [SD] 16 [8]) but low doses per injection (CD 9788 Speywood Unit [SU]) of abobotulinum toxin A) [25].

Lange et al. detected NABs by the mouse diaphragm assay in individuals with SnR who received BoNT injections for various indications [11]. NABs were detected in 17 of the 35 non-responders receiving treatment for focal spasticity. Short treatment intervals (< 3 months) and high CDs (> 6000 SU) were identified as risk factors for NAB development. The authors suggested a dose–effect relation because of the higher proportion of NABs in spasticity and dystonia than blepharospasm or hemispasm facial symptoms.

Albrecht et al. systematically sought NABs by using the mouse protection assay in 33 consecutive post-stroke patients who still responded to spasticity treatment[26]. Patients received more than 4 injections, with reinjection intervals of 12 to 13 weeks for a mean of 3.2 years with abobotulinum toxin A, onabotulinum toxin A, and incobotulinum toxin A or a switch from one to another. NABs were found in 5 patients (corresponding to a prevalence of 15% or an incidence of 4.7%/year). After conversion — 2.5 abobotulinum toxin A units equivalent to 1 onabotulinum toxin A unit or 1 incobotulinum toxin A unit — a mean dose per session > 700

unified dose units was identified as a risk factor for the development of NABs.

Occurrence and determinants of NABs in longitudinal studies

For the 9 longitudinal studies that analyzed NABs as a secondary criterion, NAB occurrence was very low: 3/1035 (0.3%) samples, with NABs found in 3 studies only [2, 16, 19], each in 1 participant only. Although these 3 participants showed SnR, the authors did not search for risk factors (dose or interval injections). Moreover, the NAB status before the study was unknown for 1 patient [16]. For the 6 longitudinal studies that did not find any NABs [15, 17, 20–23], the time interval between 2 injections ranged from 12 to 20 weeks and the mean CD of BTX was rather low for spasticity treatment: 1166 U for incobotulinum toxin A [17, 21, 22] and 3000 U for abobotulinum toxin A [23]. One study used a single injection of BoNT[15]. The CD was not given in 1 study [20]. Also, the mean (SD) number of injections was limited in the 5 longitudinal studies of NABs giving this information [15, 17, 21–23]: 3.7 (3.3) over a mean of 30 weeks. However, the CD and total number of injections individuals received was underestimated because most participants were not naïve of BoNT at inclusion in these studies [17, 19, 21–23]. The evolution of NAB over time is another factor of complexity, together with how to consider marginal NAB titrations. This situation is illustrated by the Tower study[22], which involved escalating doses of incobotulinum toxin A. At entry in the study, NABs developed in 6/133 (5%) individuals who previously received BoNT, then titres were normalized. A few other individuals showed some degree of immunogenicity at one or more times in the study, with marginal NAB titrations. The study concluded that no patients had positive hemidiaphragm assay results [22]

BoNT *formulation as a determinant of NABs*

NABs were found in 5/554 (0.9%) individuals who exclusively received onabotulinum toxin A. They were not found in the 448 individuals who exclusively received incobotulinum toxin A or the 51 who exclusively received abobotulinum toxin A [23]. NAB occurrence did not significantly differ among these formulations (Fisher exact test 4.5, p=0.1). Surprisingly, NAB prevalence was high in the 3 studies combining onabotulinum toxin A and abobotulinum toxin A [11, 24, 26] injected in different participants or successively in the same individuals. Among the 100 participants recruited by these 3 studies, 27 (27%) showed NABs. However, 2 studies [11, 26] were designed to detect NABs in participants supposed to be at high risk: those showing SnR, with long-term treatment and many injections and high CD. In the study using 3 formulations[26], the comparison of formulations was difficult given the different treatment durations for different indications (spasticity, dystonia etc.).

Causal relation between NABs and treatment non-efficacy

Participants with SnR were described in 5 of 6 studies identifying NAB-positive participants [2, 11, 16, 19, 24]. The definition of SnR to BoNT was heterogeneous, with little information given. SnR could correspond to 2 unsuccessful treatments after a previous good clinical response, as indicated by the patient [11, 24]; loss of clinical response [19]; or the absence of clinical response without further detail [2, 16].

Among the 32 participants of this systematic review who were NAB positive, 23 (72%) showed SnR, but the treatment still seemed effective in 9 (28%). Among the 41 participants with SnR in all 14 studies analyzed, 23 (56%) were NAB positive and 18 (44%) were NAB negative. From findings in these studies, we calculated the sensitivity and specificity of NAB detection in terms of non-response to treatment: the identification of non-response by NAB positivity had sensitivity of 56% and specificity of 99.6%.

However, most positive serum samples (17/32) were detected in a single study in participants with SnR [11]; half did not exhibit NABs despite detection by the gold-standard mouse hemidiaphragm technique. In contrast, 5 positive serum samples were detected in a single study of 33 participants considered to be still responding to treatment [26]. On discarding these 2 selected populations of SnR and still responding, NABs were detected in only 10/116 samples from the 12 other studies, corresponding to 6 cases of SnR. The details of the association between NABs and non-response are in Table 4. Again, the treatment was ineffective in most participants who were NAB positive (71%). In the Lange et al. study [11], 18/35 (51%) participants with SnR were NAB negative. The 13 other studies detected 1184 NAB-negative samples without SnR. Despite non-exhaustive information on the status of a BoNT responder, many patients who were NAB negative were also responders in the 14 studies analyzed for this systematic review.

Other possible influences: disease, age, geographical origin, limb or muscle injected

The 2 main conditions represented in these 14 studies were stroke in adults (91%) and cerebral palsy in children (6%). NAB positivity was found in both populations. The data presented did not allow for analyzing the effect of age on the presence of NABs. Participants of the 14 studies were almost all recruited in North America (47%) or Europe (37%), otherwise in Australia or Korea. We could not conclude on the effect of geographical origin on NAB production. The site(s) of injection did seem to affect immunogenicity. Among the 32 individuals who were NAB positive and this information was available, 27 received injections indifferently in the upper and lower limbs, 3 for only an upper-limb spasticity, and 2 for only a spasticity limited to the triceps surae.

Discussion

To our knowledge, this is the first systematic review aimed at specifically determining the prevalence of NABs in people receiving BoNT injection for limb spasticity and analyzing their possible determinants and causality in SnR. Among the 14 articles published from 2002 to 2018, 1234 serum samples were tested after BoNT injection, with NAB prevalence about 1%. NAB positivity seemed favored by long-duration therapy with high doses and a short interval between injections. NAB positivity identifying SnR had sensitivity of 56% but specificity of 99.6%. No consensual criteria were used to diagnose non-response to BTX injection.

Among the 14 studies included, the quality was globally satisfactory and the treatment protocols were homogeneous in terms of median dose injected per session (approximately 300 to 400 M-U for onabotulinum toxin A or incobotulinum toxin A and 720 SU for abobotulinum toxin A) and interval between injections (≥ 3 months). The total number of participants recruited was sufficient (1234) to support a deep analysis. Missing information was limited and we may consider that conditions were met to reach robust conclusions.

Prevalence of NABs with BoNT treatment for spasticity

NABs were detected in 1% of non-selected participants (i.e., without few individuals selected for their high risk), which is consistent with the 0.5% found specifically for limb spasticity in a review of many forms of muscle overactivity [29]. This prevalence for spasticity is lower than that reported for other indications for BoNT injection. Indeed, a prevalence of 5.9% was reported in a study examining all indications for BoNT injection for muscular hyperactivity [9]. In cervical dystonia, the prevalence ranges from 5% to 18% [5, 12, 30, 31]. The history of BTX treatment for muscular overactivity might explain these findings. The use of BTX injections for cervical dystonia preceded by several years the use of BTX injections for limb spasticity. The first studies reporting NABs after BTX injection for cervical dystonia were performed with an

old formulation of onabotulinum toxin A [32], no longer used at the time of the studies we analyzed (all published between 2002 and 2018) Thus, it is unlikely that these seminal results may be transposed today. Participants undergoing treatment for limb spasticity in our studies mostly presented hemiparesia secondary to stroke, whereas those undergoing treatment for cervical dystonia mostly presented disease of an unknown cause, possibly genetic. We cannot exclude *a priori* that this difference in nature of these two neurological conditions might play a role in NAB production.

Determinants of NAB production with BoNT treatment for spasticity

In general, studies of various muscular overactivity, and more particularly cervical dystonia in the 1990s [6, 12, 33, 34], suggested that a short interval between injections[12, 31], long-duration therapy, or a high dose per session increased the risk of immunogenicity with BTX treatment [10]. Our systematic review of spasticity strengthens this view. All cross-sectional studies for which NAB detection was the primary criterion indicated the imputability of these factors. However, this postulation remains debated because short-term satisfactory tolerance was observed in one study with escalating doses of incobotulinum toxin A in spasticity[22]. Congruently, no longitudinal study that used a low CD and a relatively long interval between injections detected any NABs. It is rare in medicine that all studies analyzed reached a similar conclusion. This conclusion becomes robust when obtained from data gathered and analyzed in a systematic review. Although the prevalence of NAB causing treatment non-response was very low, our study suggests that it could be further reduced to respect regulations and guidelines regarding doses and the time between injections. The dilution of the BoNT might also play a role in the general diffusion of the product as well as NAB production. Our systematic review did not gather enough data to analyze this factor.

The 3 available formulations of BoNT-A were well represented in this systematic review of limb spasticity. We found no difference between drugs regarding NAB prevalence. However, the prevalence of NAB was very high (27% on average) in the 3 studies combining 2 different formulations (onabotulinum toxin A and abobotulinum toxin A), in different participants or successively in the same participants [11, 24]. This finding appeals for further investigations, and quick conclusions are to be avoided because these 2 studies were designed to detect NABs in participants supposed to be at high risk.

Relation between NAB detection and SnR

This systematic review points out the lack of consensual criteria to diagnose non-responsiveness to BTX treatment. An effort should be made to list all factors that may lead to treatment failure, considering initial individualized goals. An inappropriate localization technique such as manual palpation used in isolation may also negatively affect the response to treatment [35]. Today, the use of an instrumental technique is mandatory. Too-optimistic expectations inevitably lead to deceiving the spastic person and must be distinguished from treatment failure. The indication must be correctly established for muscular overactivity corresponding to spasticity because other forms of limb hypertonia do not respond to BoNT or respond less. Histological and visco-elastic muscular changes occur over time and may reduce treatment efficacy. This situation is favored by disuse of paretic muscles, which leads to fiber shortening and accumulation of conjunctive tissue [36–39]. The greater the fibrosis involution, the less efficient the BoNT injections [40]. Comorbidities or the occurrence of other neurologic troubles may decrease the spasticity part in the disability, which explains why BoNT injections no longer lead to enhancement as much as in the past. The physician must ensure that nothing amplifies the stretch reflex of the spastic person. Such additional dysregulations of spinal reflexes are generally caused by nociceptive stimuli originating from the skin, musculoskeletal

system or viscera [41]. They frequently alter the efficacy of anti-spastic therapeutics and must be systematically sought and treated for themselves. Several years of experience with the condition and practice with BoTN injections should improve the process: acquiring the skills for this technical care, enhancing the targeting of muscles to treat and optimizing injection sites within muscles, and determining the appropriate BTX dose.

This systematic review contributes to clarifying the association between SnR and NABs. NABs identified non-response with sensitivity of 56%. Thus, immunogenicity is not the most common cause of treatment failure. The factors described above are more important. In contrast, false-positive findings were rare and NAB positivity revealed SnR with high specificity (99.6%). We suggest that in routine clinical practice, when all other possible causes of treatment failure have been discarded, NABs should be systematically sought by using tests with 100% specificity, to explain the non-response to BoNT.

Immunogenicity as the most likely cause of resistance to treatment: which strategy?

This systematic review shows that with NAB secretion against a given preparation, the preparation should not be re-injected, at least in the short term. Whether another preparation would be effective is unclear. Clinical evidence is missing to favour this strategy. Few studies analyzed the effectiveness of switching therapy from BoNT-A to another preparation of BoNT-A or another BoNT serotype, and these are only observations for spasticity. Santamato et al. reported one individual with SnR to onabotulinum toxin A, who some months later showed response to incobotulinum toxin A [42]. Because NABs were not measured and no cross-over was proposed to confirm the diagnosis, no conclusion may be drawn from this observation. In the Tower study of escalating doses of incobotulinum toxin A [22], NABs were detected at baseline in 6/133 (5%) patients who previously received BoNT, without any indication as to whether they showed response or not to BoNT before the study. The patients remained in the

study for the entire duration, with no lasting immunogenicity recorded at the end and showing response. Today, with immunogenicity and true non-response, the only recommendation is to check for NABs by using a technique that does not give false-positive results and interpret the level with respect to a given threshold for the technique used. Indeed, marginally positive results may be compatible with response to treatment[22]. In the absence of evidence dedicated to spasticity, it is impossible to recommend any therapeutic strategy.

Is there any place for the frontalis test in spasticity? This test, recommended in the field of cervical dystonia, consists of injecting a small amount of BoNT into one frontalis muscle[43]. This is a simple way to confirm for a given patient that non-response to treatment is due to the inefficacy of the drug. Only one study in our review reported the use of the frontalis test to detect treatment failure, which indicates a lack of interest for this test in spasticity treatment. This study showed a concordance, in the 3 patients tested, between the information obtained with the frontalis test and that obtained by NAB detection[24]. The accuracy and objectivity of NAB detection, which leads to direct knowledge about any immunogenicity to BoNT and its degree of severity should definitely replace the empirical frontalis test, at least in the field of spasticity.

Should we try another BoNT formulation? Of note, no study has ever compared the immunogenicity induced by different formulations nor tested a change in the BoNT formulation. Should we interrupt BoNT injections, at least in the short term? Further studies are needed to address these questions. In the meantime, one may consider biological arguments. Each formulation is a unique association of neurotoxins, complexing-proteins and excipients, all representing possible targets for antibodies[41]. One important source of immunogenicity is complexing-proteins [44]. Consequently, one cannot exclude that a given individual might secrete some antibodies against a given preparation and not against others. Therefore, with resistance to treatment due to NABs, clinicians might consider this argument to try another

preparation. The antigenicity of a BoNT preparation also depends on the amount of botulinum neurotoxin presented to the immune system, which might be determined by the specific biological activity (relation between the activity and weight of a toxin). Therefore, all BoNT products contain some biologically inactive botulinum toxin. However inactivated botulinum toxin does not exert a therapeutic effect but may act as an antigen for NABs. High immunological quality means that low antigenicity is therefore described by high specific biological activity[14]. With this pharmacological viewpoint, incobotulinum toxin A is supposed to induce lower antigenicity than other BoNT preparations [45–47]. However, in clinical practice, in the Tower study, 4% of patients exclusively using this drug showed some degree of immunogenicity at one or more times [22].

Study limitations and perspectives

Only 14 articles could be analyzed for this systematic review, with differing designs, which were globally quantified as satisfactory but heterogeneous (ranging from 0 to 28 a.u., with minimum and maximum scores 15 and 25, and only 4 studies scoring ≥ 21). The sample size was rather limited per study (only 7 studies with ≥ 100 participants). Most positive serum samples (17/32) were detected in a single study focused on SnR, with a sample size of only 35 participants [11]. If the weight of this study is important in terms of the prevalence of NABs in SnR, we do not think the study biased the results because the rate of false-positive findings was similar with and without the study. Only the Tower study detected NABs at entry[22], which is insufficient to give a global view about changes in immunogenicity (appearance or disappearance) with an intervention. Future studies should address this issue. Many patients were injected before entry in studies and missing information about injection doses and intervals before studies might have biased our analyses and conclusions. In fact, many studies give this information about the CD and mean interval of injections, before and after inclusion.

When this information is lost, there is no reason to think that it affects one group more than the other: low versus high CDs; long versus short intervals.

We hope that our efforts to precisely collect this piece of information, together with our efforts to objectively reduce any biases, limited sources of errors regarding the effect of CDs and time intervals on the occurrence of NABs. Probably a strength of our study is being totally independent of industry, especially on that question.

However because of these limitations, one needs to be prudent regarding the generalization of the results of this systematic review. The results must be considered rather a starting point for a specific line of research for limb spasticity.

Conclusion

Among 1234 serum samples tested, NAB prevalence with BoNT injections for limb spasticity was estimated at 1% to 2%, according to various scenarios, with no difference between BoNT formulations. This rate is much lower than that reported for dystonia. Immunogenicity is not the most common cause of non-responsiveness to treatment, which is consistent with the general feeling that immunogenicity and SnR is not a real problem with current BoNT formulations. NABs should be searched only when no other cause explains an apparent resistance to treatment, which indeed would need to be defined by consensual criteria. A test with 100% specificity is recommended to quantify NABs. If immunogenicity is the most likely cause of non-response to treatment, some biological arguments suggest trying another BoNT, but no clinical evidence supports this strategy today.

Conflict of interest. None

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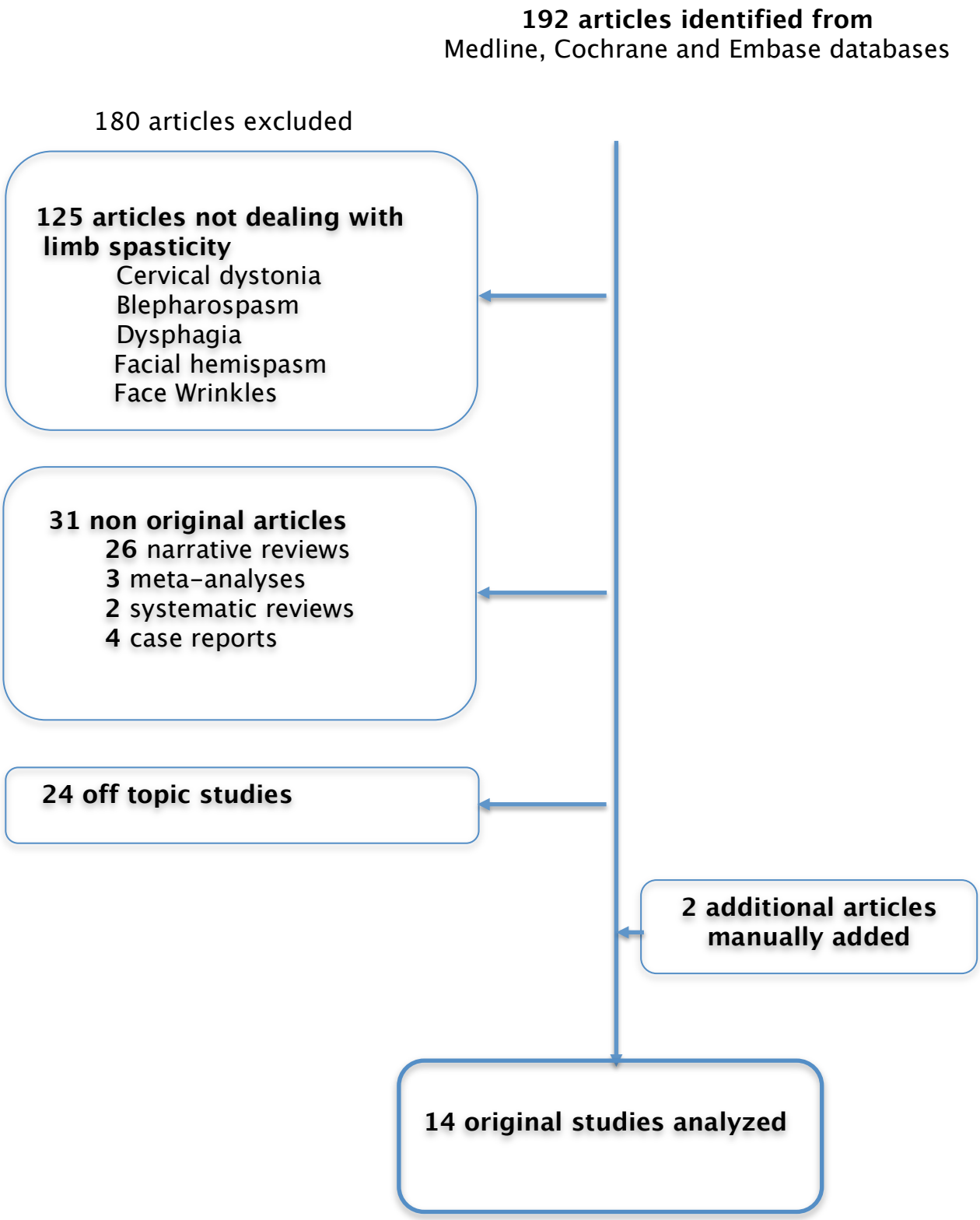

Figure 1. Flow of articles in the systematic review.

Table 1. Quality analysis form used in the systematic review. For questions 1,2,4,7,8,10,11,12,13 and 14 the scoring was as follows: 0, no description; 1, limited description; 2, good description. BoNT: botulinum toxin, NAB: neutralizing antibody, SnR: secondary non-response.

Introduction	
Q1	Is the scientific context clearly explained?
Q2	Are the objectives clearly stated?
Methods	
Q3	What is the design of the study? (0 retrospective; 1 prospective without control group; 2 RCT)
Q4	Are inclusion criteria and selection of participants clearly explained?
Q5	Sample size (0 if < 20, 1 between 20-100, 2 >100)
Q6	Is the influence of dose or type of BoNT controlled? (0 no, 1 only 1 criteria, 2 both)
Q7	Is the method (validity) of NAB measurement described?
Q8	Are statistical analyses appropriate?
Results	
Q9	Is there a flow chart? (0 yes, 2 no)
Q10	Are subjects' characteristics described?
Q11	Are the results interpretable?
Q12	Is the link between SnR and NAB detection analysed?
Q13	Are study limitations discussed?

Table 2. Quality assessment analysis. The 4 studies analyzing NAB as a main criterion are presented first, then the 9 others, by chronologic order.

Articles	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Total
Albrecht et al. 2019	2	2	1	2	2	2	2	2	0	2	2	0	2	2	23
Oshima et al. 2017	2	2	2	2	1	0	2	2	0	2	2	0	2	2	21
Backeit et al. 2012	2	2	0	1	0	0	2	0	0	1	0	0	1	1	10
Lange et al. 2009	2	2	0	1	2	0	2	1	0	0	1	2	0	2	15
Muller et al. 2009	2	2	1	1	1	2	2	0	0	1	1	1	1	2	17
Wissel et al. 2017	2	2	1	2	2	2	1	2	0	2	2	0	1	2	21
Jang et al. 2014	1	1	1	1	1	2	1	2	0	2	1	0	2	2	17
Kanovski et al. 2011	2	1	1	1	2	2	1	1	0	2	2	0	1	2	18
Kanovski et al. 2009	2	1	2	1	2	2	1	2	0	2	1	0	1	2	19
Elovic et al. 2008	2	2	1	2	2	2	1	1	2	2	2	2	2	2	25
Backeit et al. 2004	2	1	2	1	1	2	1	0	0	2	1	0	2	2	17
Brashear et al. 2004	2	1	2	2	0	2	0	2	0	2	0	0	2	2	17

Table 3. Characteristics of the 14 selected studies selected for the systematic review. The 4 studies analyzing NAB as a main criterion are presented first, and the 9 others second, by chronologic order from the earliest to oldest. NAB, neutralizing antibody. PD and CBRS, Patient's Disability and Care giver Burden Rating Scale. Muscle acronyms: BB, biceps brachii; BR, brachioradialis; B, brachialis; FCR, flexor carpi radialis; FCU, flexor carpi ulnarisl FDS, flexor digitorum superficialis; FDP, flexor digitorum profundus; PR, pronator quadratus; PT, pronator teres; FPL, flexor pollicis longus; AP, adductor pollicis; GM, gastrocnemius medialis.

Author, Year	Study design	No. of participants	Age, years, mean (SD)	Neurological conditions	Principal study criteria	Technique for measuring BTX neutralizing antibodies (NABs)	Injection sites
Detection of NAB as main criterion							
Albrecht et al. 2019	Prospective, observational cross-sectional study	33	62 (14)	Various conditions of hypertonia including focal spasticity in post-stroke patients	Measure of BoNT antibodies in patients still responding to treatment	Mouse hemidiaphragm assay	Spastic muscles of upper and lower limbs without details
Oshima et al. 2017	Randomized controlled trial	38	3.3	Cerebral palsy	Measure of BoNT antibodies 2 groups: BoNT injection annually/triannually	Mouse protection bioassay	Triceps surae
Bakheit et al. 2012	Retrospective, observational	18	46.6	Various conditions: stroke, cerebral palsy, multiple sclerosis, familial spastic paraparesis	Measure of BoNT antibodies Analysis of risk factors	Mouse protection bioassay	Spastic muscles of upper and lower limbs without details
Lange et al. 2009	Multicenter, prospective, observational	35	-	Various conditions of hypertonia including focal spasticity	Measure of BoNT antibodies in secondary non-response	Mouse hemidiaphragm assay	Spastic muscles without details
Muller et al. 2009	Prospective, observational	42	56.7 (11.9)	Various conditions: stroke, multiple sclerosis, head trauma	Measure of BoNT antibodies in patients after long-term treatment with BoNT	Mouse hemidiaphragm assay	Spastic muscles without details
Detection of NAB as secondary criterion							
Wissel et al. 2017	Multicenter, prospective, non-randomized, single-arm, open-label, dose-titration	155	53.7 (13.1)	Chronic spastic hemiplegia due to any cerebral lesion	Safety of increasing doses Systematic registration of adverse effects Efficacity Spasticity	Mouse hemidiaphragm assay	All muscles implied in spastic clinical patterns in upper and lower limbs

					Improvement on personalized goals Measure of BoNT antibodies		
Jang et al. 2014	Prospective, single blind	38	5.5 (2.7)	Cerebral Palsy	Efficacity Spasticity Improvement on personalized goals Measure of BoNT antibodies	Bridging ELISA+competitive ELISA+ Mouse protection assay	GM
Kanovski et al. 2011	Multicenter, prospective, non-randomized, open-label	145	55.7 (12.1)	Chronic stroke	Efficacity Spasticity (MAS) Improvement on personalized goals Safety Adverse events Measure of BoNT antibodies	Mouse hemidiaphragm assay	Elbow flexors, wrist flexors, finger flexors, forearm pronators, thumb flexors
Kanovski et al. 2009	Multicenter, randomized, placebo-controlled, double-blind	148	58.1 (10.2)	Chronic stroke	Efficacity Spasticity (MAS) Improvement on personalized goals Measure of BoNT antibodies	Mouse hemidiaphragm assay	Elbow flexors (BR+BB+B), wrist flexors (FCU + FCR), finger flexors (FDS+FDP), forearm pronators (PQ+PT), thumb flexors
Elovic et al. 2008	Multicenter, open label, repeated dose	279	58 (13)	Chronic stroke	Efficacity Spasticity Improvement on personalized goals Quality of life Safety Adverse events Measure of BoNT antibodies	Mouse protection assay	Elbow flexor, wrist flexor, finger flexor, thumb flexor
Bakheit et al. 2004	Multicenter, open label, blind evaluation	51	56 (11.5)	Subaccute stroke	Efficacity of repeated injections Spasticity	Standard mouse lethality assay (LD50)	BB, FDS, FDP, FCU, FCR

					Range of motion Improvement on personalized goals Safety Adverse events Measure of BoNT antibodies		
Brashear et al. 2004	Double-blind, placebo controlled, followed by an open label phase	15	55 (17)	Chronic stroke	Efficacity Spasticity Improvement on personalized goals Safety Adverse events Measure of BoNT antibodies	Mouse neutralization studies (no details)	BB, FCR, FCU, FDS, FDP
Gordon et al. 2004	Open label	111	61.5 (14.1)	Chronic stroke	Efficacity Spasticity Functional evaluation Safety Adverse events Measure of BoNT antibodies	Mouse protection assay	FCR, FCU, FDP, FDS, FPL, AP
Brashear et al. 2002	Multicenter, randomized, double-blind, placebo controlled	126	62	Chronic stroke	Efficacity Spasticity Functional evaluation Improvement on personalized goals Safety Adverse events Measure of BoNT antibodies	Mouse protection assay	FCR, FCU, FDP, FDS, FPL, AP

Table 4. Summary of findings on risk factors to develop NABs and association between NABs and secondary non-response. The 4 studies analyzing NAB as a main criterion are presented first, and the 9 others second, by chronologic order. Concerning potential risk factors, cumulative dose was not described in 3 studies nor was the interval between injections in 1 study. The total number of injections was not controlled in 5 studies in which participants were not naïve of BTX treatment at inclusion. *Toxin brands: Abobotulinumtoxin A (Dysport®), Incobotulinumtoxin A (Xeomin®,) Onabotulinumtoxin A (Botox®), Botulinux A toxin (Meditoxin®), Rimabotulinumtoxin B (Myobloc®). MU-E: equivalent mouse unit*

Author, Year	Toxin brand name	Cumulative dose (CD) Mean dose per treatment (MD) Last dose (LD)	Total no. of injection cycles Duration of therapy (DT)	Interval between injections	Association between neutralizing antibodies (NABs) and non-response
Detection of NAB as main criterion					
Albrecht et al. 2019	Abobotulinumtoxin A Onabotulinumtoxin A Incobotulinumtoxin A (conversion ration 2.5/1)	CD: not indicated MD: 957 unified dose units LD: not indicated	At least 4 injections DT: 3.2 years	12-13 weeks	5 NAB samples detected in 33 patients still responding to treatment (prevalence 15%)
Oshima et al. 2017	Onabotulinumtoxin A	CD: not indicated MD: not indicated (max 12 U/kg) LD: not indicated	2 groups: 2 or 6 injections	2 groups: 4 months or 1 year	NABs detected in 2 participants treated triannually Clinical response was not described
Bakheit et al. 2012	Abobotulinumtoxin A Onabotulinumtoxin A (conversion ration 3/1)	CD indicated for Abobotulinumtoxin A: 9788 (6091) MD: not indicated LD: not indicated	16 (8) injections DT: 93 months (35)	103 (63.6) to 386 (188) days	No antibodies detected even in 2 non-responders
Lange et al. 2009	Abobotulinumtoxin A (69%) Onabotulinumtoxin A (13%) Both (7%) No information (11%)	Documented in 251 patients receiving Abobotulinumtoxin A: CD: 10700 SU MD: 750 SU LD: not indicated	Number of injections: not indicated Mean duration therapy before failure: - Abobotulinumtoxin A: 41 months - Onabotulinumtoxin A: 45 months	Documented for 251 patients (Abobotulinumtoxin A): 3.1 months	Among 35 non-responders participants treated for focal spasticity 17 had NABs

Muller et al. 2009	Onabotulinumtoxin A (21/42) Abobotulinumtoxin A (14/42) Onabotulinumtoxin A and Abobotulinumtoxin A (7/42)	CD (MU-E): - Onabotulinumtoxin A: 5.4 (2.3) - Abobotulinumtoxin A: 4.7 (2.5) MD (MU-E) = 399 (134) LD: not indicated	14.2 (6.1) injections DT: 4.5 (1.8) years	4.1 (1.2) months	NABs detected in 5 of the 42 participants 3 were secondary non-responders
Detection of NAB as secondary criterion					
Wissel et al. 2017	Incobotulinumtoxin A	CD: 2000 U MD: not indicated LD: 800 U	3 injections with increasing dose during the study No data on previous injections DT: not indicated	12-16 weeks	No antibodies detected
Janget al. 2014	Botulinux A toxin	CD: not indicated MD: 4U/kg LD: not indicated	1 to > 6 injections DT: not indicated	Not indicated	No antibodies detected
Kanovski et al. 2011	Incobotulinumtoxin A	CD: 1197 (450.8) Median dose: 400 U LD: 339.4 (87.8)	6 injections of Incobotulinumtoxin A in prospective analysis + 3.4 previous injections with Onabotulinumtoxin A or Abobotulinumtoxin A DT: not indicated	12 weeks	No antibodies detected
Kanovski et al. 2009	Incobotulinumtoxin A	Prospective analysis with 1 injection CD: not indicated MD: 307 U LD: not indicated	First injection for 75% of participants	Not applicable	No antibodies detected
Elovic et al. 2008	Onabotulinumtoxin A	CD: not indicated MD: not indicated LD: not indicated For each injection: min 200 U and max 400 U	1 to 5 injections in prospective analyse No data on previous injection DT: 54 weeks	12 weeks	NABs detected in 1 participant before the 5th injection who was a secondary non-responder

Bakheit et al 2004	Abobotulinumtoxin A	CD: 3000 U max in prospective analysis MD: not indicated LD: not indicated	3 injections in prospective analysis No data on previous injection DT: 36-48 weeks	12-20 weeks	No antibodies detected
Brashear et al. 2004	Rimabotulinumtoxin B	CD: 20 000 U MD: 10 000 U LD: 10 000 U	2 injections DT: 28 weeks	16 weeks	No antibodies detected
Gordon et al. 2004	Onabotulinumtoxin A	CD: not indicated MD = 220 U LD: not indicated	2,8 injections DT: not indicated	12-15 weeks	NABs detected in 1 participant who was non-responder
Brashear et al. 2002	Onabotulinumtoxin A	A single injection: MD: 221 U Maximum dose = 240 U	Not applicable	Not applicable	NABs detected in 1 participant who was non-responder

SU, Speywood unit