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Systematic Review and Meta-Analysis of Secondary Treatment Failure and Immunogenicity With Botulinum Neurotoxin A in Multiple Indications

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ABSTRACT

Background: Botulinum neurotoxin A (BoNT-A) is recommended for the treatment of cervical dystonia (CD), spasticity, and blepharospasm. Some patients treated with BoNT-A have been reported to develop neutralizing antibodies (NAbs) against BoNT-A, which may result in reduced efficacy and, in some cases, secondary treatment failure (STF). Our aim was to investigate the incidence of STF and NAb positivity after treatment with one of three commercially-available BoNT-A formulations.

Methods: A systematic review and meta-analysis of STF and/or NAb positivity after treatment with abobotulinumtoxinA, incobotulinumtoxinA, or onabotulinumtoxinA in patients with CD, spasticity, or blepharospasm was conducted using PubMed, Embase, and Google Scholar.

Results: Twenty-nine unique studies reported in 29 publications assessed NAb positivity and were included. The meta-analysis showed that the proportions of patients developing STF were significantly higher after treatment with abobotulinumtoxinA or onabotulinumtoxinA than with incobotulinumtoxinA for CD or spasticity. Depending on the antibody test used, the proportions of patients developing NAbs were also significantly higher after treatment with abobotulinumtoxinA or onabotulinumtoxinA than with incobotulinumtoxinA for CD or spasticity. When data for all indications were pooled, proportions of NAb-positive patients were numerically higher with increasing mean doses of abobotulinumtoxinA or onabotulinumtoxinA. No patients treated exclusively with incobotulinumtoxinA were found to have developed immunogenic STF or persistent NAbs.

Conclusions: The risk of developing STF and NAbs appears to vary with indication and BoNT-A formulation. When the efficacy and safety of formulations are comparable, incobotulinumtoxinA may be recommended to avoid developing STF and immunogenicity, particularly for patients requiring higher doses and repeated treatments.

Equal contribution: Uwe Walter and Phillipp Albrecht first authors, and Warner Carr and Harald Hefter last authors.

1 | Introduction

Botulinum neurotoxin A (BoNT-A) is a recommended treatment option for several neurological and muscular conditions, including spasticity [1, 2], cervical dystonia (CD) [3, 4], sialorrhea [5, 6], and blepharospasm [4], and is widely used for esthetic procedures [7]. AbobotulinumtoxinA, incobotulinumtoxinA, and onabotulinumtoxinA are commercial preparations of BoNT-A approved for the treatment of CD, spasticity, and blepharospasm that have been available for more than 15 years.

The dose of BoNT-A, and the frequency and duration of treatment can vary greatly between indications, ranging from low single doses for esthetic procedures to higher, repeated doses for CD, in some cases for the lifetime of the patient. As with other biological drugs, long-term treatment with BoNT-A can lead to the development of neutralizing antibodies (NAbs). In some patients, this immunogenicity against the botulinum neurotoxin can limit the efficacy of established treatments, necessitating increased treatment doses to maintain efficacy, or causing secondary treatment failure (STF) [8–11]. The immunogenicity potential of a BoNT-A varies based on both the amount of active neurotoxin protein and the amount of nontherapeutic accessory clostridial proteins in each formulation [8, 10, 11]. Although not therapeutically active, accessory proteins can induce an immune response and facilitate the development of NAbs [8, 12, 13]. For example, the first commercially available formulation of onabotulinumtoxinA, which contained a relatively high quantity of accessory proteins, was associated with a higher risk of immunogenicity than the currently marketed formulation, which superseded it in 1997 [10]. The risk of developing immunogenicity to BoNT-A may be increased with dose per treatment session [11, 14] and with greater length of continuous treatment [15]. Inter-injection intervals also play a role, with shorter intervals potentially leading to earlier treatment failure [16]. Two types of assays are commonly used in clinical studies for the detection of NAbs: the mouse protection assay and the mouse hemidiaphragm assay (MHDA). The MHDA is at least five times more sensitive than the mouse protection assay [10, 17]. This is because the mouse protection assay uses live mice as an indirect intoxication model and the test sample is exposed to complex pharmacokinetics, whereas the MHDA involves direct testing on an isolated respiratory muscle, reducing variability between replicates and improving sensitivity [18, 19]. Routine NAb testing is uncommon in clinical practice, but clinicians can look for clinical hints of NAb positivity, which include the need to increase the dose of BoNT and variability of BoNT effect over time after repeated injections [20].

The aims of this paper were to (1) conduct a systematic literature review to identify studies assessing immunogenicity after treatment with three commercial BoNT-A preparations for which long-term data have been published, in three therapeutic indications (CD, spasticity, and blepharospasm), (2) extract data on the reported number of patients with NAbs and the number of patients with STF, and (3) perform a meta-analysis of these data and explore whether the proportions of patients with STF or NAbs vary between different patient populations and BoNT-A formulations.

2 | Methods

2.1 | Search Strategy

PubMed and Embase were queried with the following search string on 9 April 2024: (*abobotulinumtoxina* OR *dysport* OR *onabotulinumtoxinA* OR *botox* OR *incobotulinumtoxinA* OR *xeomin* OR "*botulinum neurotoxin*" OR *BoNT-A* OR "*botulinum toxin*" OR *BTX-A*) AND (*tolera** OR *immunogenicity* OR *antibod** OR *NAb*). No limits were applied. In addition, multiple searches of Google Scholar were performed using the formula [*short name for toxin**] OR [*brand name for toxin*] AND [*condition*] AND *antibodies* OR *immunogenicity* OR *NAb*. For example, the search string for abobotulinumtoxinA in spasticity was *abo** OR *dysport* AND *spasticity* AND *antibodies* OR *immunogenicity* OR *NAb*. The searches were ordered by "most relevant" according to the search engine, and the first 40 results were screened for any eligible publications not already identified in the PubMed and Embase searches. Google Scholar was included as unlike PubMed or Embase which only search the title, abstract and author information, it also searches the main text of publications and can therefore identify additional relevant papers missed by PubMed or Embase.

Inclusion criteria were as follows: toxin(s) identifiable as abobotulinumtoxinA, incobotulinumtoxinA, or 'new' formulation onabotulinumtoxinA; data reported on assessment of NAb; and treatment of CD, spasticity, or blepharospasm. The following publications were excluded: reports on original formulation onabotulinumtoxinA (excluded wherever possible, based on study dates and relevant information given in the respective publications); reviews, correspondence, guidelines or consensus statements, case reports/case series, preclinical/in vitro studies, studies that exclusively recruited patients with STF, studies investigating other therapeutic or esthetic indications, studies where type of first-line BoNT-A is unknown, studies where BoNT-A was not the currently approved version (e.g., formulations provided by a research laboratory), or publications that pooled results for different types of BoNT-A into a single group. Studies that only included patients responding to BoNT-A were excluded from the STF analysis.

2.2 | Data Analysis

Methodology, data on STF and NAb positivity by first-line BoNT formulation and dose, and patient demographic data were extracted from each study by two independent reviewers (LL and DM) and verified by a third reviewer (ADI).

Extracted data were pooled by the first-line BoNT-A formulation that patients received. Only data that could be fully attributed to the first-line BoNT-A formulation were included; in patients receiving more than one BoNT-A formulation, data reported after the patient switched formulation were excluded from analysis. Regarding NAb testing methods, data were pooled per treatment formulation for all NAb testing types, regardless of sensitivity, as well as a subgroup of studies that used the MHDA to assess NAb positivity (either for all patients or as confirmation of a less sensitive test).

Subgroup analyses were performed for the three included indications (spasticity, CD, and blepharospasm) as well as by mean dose per injection cycle, regardless of indication. Where a range of doses was reported in a study, the average was calculated.

2.3 | Meta Analysis

Meta-analysis methods were used to pool together the results from different studies within each formulation; the outcome in all analyses was the occurrence of STF or whether the patient was NAb positive or negative (binary outcomes) and only studies reporting STF data were included in the meta-analysis of STF occurrence. The DerSimonian–Laird random-effects method was used for the analyses, regardless of the degree of heterogeneity between the study results. The Freeman–Tukey double arcsine transformation was performed before each analysis. This was used to stabilize the variances as the percentage of positive patients was close to zero and one, and a Normal approximation to the binomial distribution did not hold. The Freeman–Tukey double arcsine transformation was chosen as it is the preferred method for analysis of prevalence proportions [21].

The difference in occurrence of STF or NAb positivity between the three formulations was assessed by the significance of the test for heterogeneity between groups. Initially, the overall comparison between the three formulations was examined. Subsequently, comparisons were made between pairs of formulations. Separate analyses were conducted for studies using any antibody test and for the subset of studies specifically using MHDA. An additional set of meta-analyses examined the effect of mean dose on the occurrence of NAb positivity; dose groups were calculated as tertile ranges for all mean doses reported per formulation. All statistical analyses were performed using Stata (version 15.1; StataCorp).

3 | Results

3.1 | Literature Search Results

A summary of included publications by indication is provided in Table 1, and the systematic review process is illustrated in Figure S1. NAb data were reported in 12 studies in patients treated for CD, 15 studies in subjects treated for spasticity, and three studies in patients treated for blepharospasm. The studies were not assessed for quality or bias as immunogenicity was not the focus or primary outcome of any of the identified studies.

3.2 | Secondary Treatment Failure Definitions

Definitions for STF varied considerably between studies and were not defined at all in some cases (Table 1). For the purposes of this study, all definitions were considered comparable to include as many studies as possible in the analysis. Approximately two thirds (19/29) of the included studies reported STF, and the majority of these studies used MHDA testing to assess immunogenicity. None of the three blepharospasm studies reported STF.

3.3 | STF Findings

The proportions of patients with STF, irrespective of NAb status, were numerically higher in patients with CD than in those with spasticity. In total, 9% of all patients with CD treated with first-line abobotulinumtoxinA and 3% of patients with CD treated with first-line onabotulinumtoxinA had STF. The proportions of patients with spasticity and STF were numerically lower than those with CD (5% of patients receiving first-line abobotulinumtoxinA and 0.4% of patients receiving first-line onabotulinumtoxinA). Interestingly, no reports of STF in patients treated exclusively with incobotulinumtoxinA for CD or spasticity were identified (Figures 1 and S2). This difference, of higher proportions of patients reported with STF after treatment with abobotulinumtoxinA or onabotulinumtoxinA compared with incobotulinumtoxinA, was significant in patients with CD ($p < 0.001$ and $p = 0.03$, respectively). The higher proportion of STF with abobotulinumtoxinA versus incobotulinumtoxinA in patients with spasticity was also significant ($p = 0.01$).

3.4 | Immunogenicity Testing

We identified 8/12 studies in CD, 9/15 studies in spasticity, and 3/3 studies in blepharospasm that used the MHDA. The majority of studies using the highly sensitive MHDA (19/29) were studies of incobotulinumtoxinA (Table 1).

3.5 | Immunogenicity Findings

Figures 2 and S3 show the proportions of patients with CD who were NAb positive following treatment with BoNT-A. As with STF, there was a numerically higher proportion of NAb-positive patients receiving abobotulinumtoxinA as first-line treatment (2%) than patients receiving incobotulinumtoxinA (0%) or onabotulinumtoxinA (1.5%) as first-line treatment (Figure 2A). When studies that only used MHDA were included, the proportions increased to 3.6% and 2.4% for abobotulinumtoxinA and onabotulinumtoxinA, respectively. The proportions of patients with CD who were NAb positive were significantly higher in the abobotulinumtoxinA or onabotulinumtoxinA group versus the incobotulinumtoxinA group ($p = 0.01$ and $p = 0.02$, respectively) (Figure 2B).

Figures 3 and S4 show the proportions of patients with spasticity who were NAb positive following treatment with BoNT-A. Considering all studies, regardless of the assays used for NAb testing, the proportion of NAb-positive patients receiving abobotulinumtoxinA (0.4%) was similar to those receiving onabotulinumtoxinA (1.2%) as first-line BoNT-A, whereas no patients receiving incobotulinumtoxinA exclusively were persistently NAb positive. When only MHDA testing was included, the proportions of positive patients increased to 2.8% for abobotulinumtoxinA and 2.1% for onabotulinumtoxinA. The proportion of NAb-positive patients with abobotulinumtoxinA was

TABLE 1 | Summary of included publications[a].

Study	Type of study	Toxin(s) investigated (no. of participants potentially tested for NAbs)	Participants	Most sensitive[b] NAb testing method used	Definition of secondary treatment failure	Inclusion criteria (relevant to immunogenicity)
Cervical dystonia						
Mohammadi 2009 [22]	Retrospective	Abo ($n=163$). Ona ($n=44$)	Adults	MHDA	Not defined	Patients had only received one formulation of BoNT before or during observation period
Evidente 2013 [23]	Randomized, double-blind main period and extension	Inco ($n=82$). Switched from unknown BoNT to inco ($n=132$)	Adults	MHDA	Not assessed	BoNT-naïve and experienced patients were eligible. Immunogenicity results specified whether patients were BoNT naïve or experienced at baseline
Brans 1995 [24]	Prospective	Abo ($n=41$)	Adults	MPA	More than 50% of the treatments failed to produce a relevant improvement on the 10-point anchored visual analogue scale	Patients had only received one formulation of BoNT before or during observation period
Truong 2005 [25]	Randomized, double-blind, placebo-controlled	Switched from ona to abo ($n=37$)	Adults	MPA	Not achieving a decrease in TWSTRS total score of at least 30% and at least 10 points	BoNT-naïve and experienced patients were eligible. Immunogenicity results specified whether patients were BoNT naïve or experienced at baseline. Individuals were excluded if the study physician suspected secondary non-responsiveness
Hefter 2016 [26]	Observational	Abo ($n=128$). Ona ($n=36$). Switched from unknown BoNT to inco ($n=10$)	Adults	MHDA	Not assessed (response at time of NAb test was an inclusion criterion)	Patients were included if they had treatment response after at least 10 injections without interruption within the previous 2–3 years

(Continues)

TABLE 1 | (Continued)

Study	Type of study	Toxin(s) investigated (no. of participants potentially tested for NAbs)	Participants	Most sensitive[b] NAb testing method used	Definition of secondary treatment failure	Inclusion criteria (relevant to immunogenicity)
Dressler 2013 [27]	Prospective, open-label, single-arm, multicentre	Inco ($n=51$). Switched from abo to inco ($n=14$). Switched from ona to inco ($n=11$)	Adults	MHDA	Not defined	BoNT-naïve and experienced patients were eligible. Pre-treated patients were required to have a stable response at recruitment
Brin 2008 [28]	Prospective, open-label, observational	Ona ($n=326$)	Adults	MPA	Clinical resistance indicated by Frontalis Antibody Test or Unilateral Brow Injection	Patients had to be BoNT naïve to be eligible for inclusion
Benecke 2009 [29]	Open label	Inco ($n=50$). Switched from unknown BoNT to inco ($n=50$)	Adults	MHDA	Not defined	BoNT-naïve and experienced patients were eligible. All patients were negative for NAbs at baseline
Jochim 2019 [30]	Retrospective	Abo ($n=209$). Ona ($n=135$)	Adults	Not specified which method was used	Positive NAb test or extensor digitorum brevis test	BoNT-naïve and experienced patients were eligible. Two-thirds of patients were BoNT naïve at study entry
Hefter 2022 [15]	Retrospective	Inco ($n=34$). Switched from abo to inco ($n=48$). Switched from ona to inco ($n=11$)	Adults	MHDA	Confirmed systematic worsening of Tsui score over two treatment cycles of at least 3 TSUI score points before treatment switch and lack of symptom reduction reported by the patient during these 2cycles	BoNT-experienced patients were eligible. Patients could have switched from one BoNT to another due to inadequate response

(Continues)

TABLE 1 | (Continued)

Study	Type of study	Toxin(s) investigated (no. of participants potentially tested for NAbs)	Participants	Most sensitive[b] NAb testing method used	Definition of secondary treatment failure	Inclusion criteria (relevant to immunogenicity)
Hefter 2014 [31]	Retrospective	Abo ($n = 568$)	Adults	MHDA	Partial secondary treatment failure: (1) subject previously had good treatment response (when the difference between Tsui baseline score and Tsui score determined at injection session number) χ ($\chi \geq 2$) is ≥ 3 points, and (2) a patient's Tsui score systematically worsens in spite of maintenance or increase of dose starting at injection session number y ($y > \chi$) during BoNT-A treatment, and (3) the patient had reported that the last two injections had been less effective than the previous ones. A systematic worsening of the Tsui score was defined as an increase in Tsui scores for more than two points over two treatment cycles (three consecutive Tsui scores). This definition is based on at least four Tsui scores being determined during treatment with three or more consecutive BoNT-A injections	Eligible patients could only have received abo
Bigalke 2001 [32]	Prospective	Abo ($n = 8$)	Adults	MHDA	Not assessed	Eligible patients could have received long-term treatment with BoNT
Spasticity						

(Continues)

TABLE 1 | (Continued)

Study	Type of study	Toxin(s) investigated (no. of participants potentially tested for NAbs)	Participants	Most sensitive[b] NAb testing method used	Definition of secondary treatment failure	Inclusion criteria (relevant to immunogenicity)
Bakheit 2004 [33]	Prospective, open label	Abo ($n = 32$)	Adults with PSS	Mouse lethality assay	Not assessed	Eligible patients could not have received treatment with BoNT-A in the 90 days preceding the study
Delgado 2021 [34]	Randomized repeat-treatment	Abo ($n = 72$). Switched from unknown BoNT to abo ($n = 138$)	Children with CP	MPA	Not assessed	Previous treatment with BoNT must have been > 6 months before study entry in study limb; > 3 months in other injection sites
Gracies 2017 [35]	Double blind, randomized, placebo controlled, single cycle	Abo ($n = 226$). Switched from unknown BoNT to abo ($n = 126$)	Adults with spastic hemiparesis causing gait dysfunction	MPA	Not assessed	64% of patients were BoNT naïve at baseline
Kanovský 2009 [36]	Assessor blinded, randomized, parallel group	Abo ($n = 214$)[c]	Children with CP	MHDA	Not assessed	Eligible participants had not received BoNT within 9 months before study entry
Oshima 2017 [37]	Randomized clinical trial	Ona ($n = 38$)	Children with CP	MPA	Not assessed	Eligible patients were BoNT naïve at study entry
Elovic 2008 [38]	Open label, repeated dose	Ona ($n = 224$)	Adults with PSS	MPA	Frontalis Type A test	BoNT-naïve and experienced patients were eligible, but the only acceptable formulation was ona (second generation)
Gordon 2004 [39]	Open-label extension	Ona ($n = 110$)	Adults with PSS	MPA	Not defined	Eligible patients were BoNT naïve at study entry (main period)

(Continues)

TABLE 1 | (Continued)

Study	Type of study	Toxin(s) investigated (no. of participants potentially tested for NAbs)	Participants	Most sensitive[b] NAb testing method used	Definition of secondary treatment failure	Inclusion criteria (relevant to immunogenicity)
Müller 2009 [40]	Observational	Abo ($n=14$). Ona ($n=21$)	Adults with spasticity of various aetiologies	MHDA	Not defined	Eligible patients had received BoNT for at least 2 years before study entry
Masakado 2020 [41]	Randomized, double blind, placebo controlled	Inco ($n=67$)	Adults with PSS	MHDA	< 1-point reduction in MAS score	Eligible patients were BoNT naïve at baseline or had received ona at least 16 weeks before study entry
Masakado 2022 [42]	Randomized, double blind, placebo controlled	Inco ($n=95$). Switched from ona to inco ($n=107$)	Adults with PSS	MHDA	< 1-point reduction in MAS score	A washout period of at least 16 weeks was required between pretreatment with any BoNT for any indication and the screening visit
Kaňovský 2011 [43]	Assessor blinded, parallel group, open-label extension	Inco ($n=145$)	Adults with PSS	MHDA	Worse than baseline or no response on 4-point impression of treatment effect scale	Eligible participants had not received BoNT within 9 months before study entry (main period)
Marciniak 2019 [44]	Open-label extension	Inco ($n=296$)	Adults with PSS	MHDA	Not defined	Eligible patients could not have received treatment with any BoNT formulation in any body region for any indication in the previous 12 months before study entry (main period)
Wissel 2017 [45]	Prospective, single arm, dose titration	Inco ($n=22$). Switched from unknown BoNT to inco ($n=133$)	Adults with spasticity due to cerebral causes	MHDA	Not defined	BoNT-naïve and experienced patients were eligible

(Continues)

TABLE 1 | (Continued)

Study	Type of study	Toxin(s) investigated (no. of participants potentially tested for NAbs)	Participants	Most sensitive[b] NAb testing method used	Definition of secondary treatment failure	Inclusion criteria (relevant to immunogenicity)
Kaňovský 2022 [46]	Open label, noncontrolled	Inco ($n=54$). Switched from unknown BoNT to inco ($n=66$)	Children with CP	MHDA	Not defined	BoNT-naïve and experienced patients were eligible; 55% were previously treated at study entry
Dressler 2015 [47]	Prospective, non-interventional	Inco ($n=54$)	Adults with various forms of spasticity	MHDA	Initially sufficient BoNT effects, subsequently shortened durations of action and complete lack of BoNT efficacy on three subsequent BoNT injection series	BoNT-naïve and experienced patients were eligible
Blepharospasm						
Jankovic 2011 [48]; Truong 2013 [49]	Double blind, parallel group, placebo controlled including open-label extension	Switched from ona to inco ($n=102$)	Adults	MHDA	Not assessed	Eligible patients had a stable therapeutic response to the last two consecutive injections with ona, administered ≥10 weeks before trial entry (main period)
Bigalke 2001 [32]	Prospective	Abo ($n=8$)	Adults	MHDA	Not assessed	Eligible patients could have received long-term treatment with BoNT
Mitsikostas 2020 [50]	Prospective, double-blind, placebo-controlled, randomized, parallel-group, multicentre study comprising a 6- to 20-week main period and a 6- to 20-week open-label extension	Inco ($n=41$)	Adults	MHDA	Not defined, although response assessed using Investigator's Global Assessment of Efficacy and Patient Evaluation of Global Response	Eligible patients were toxin naïve (defined as ≥12 months without receiving any formulation of BoNT for the treatment of blepharospasm)

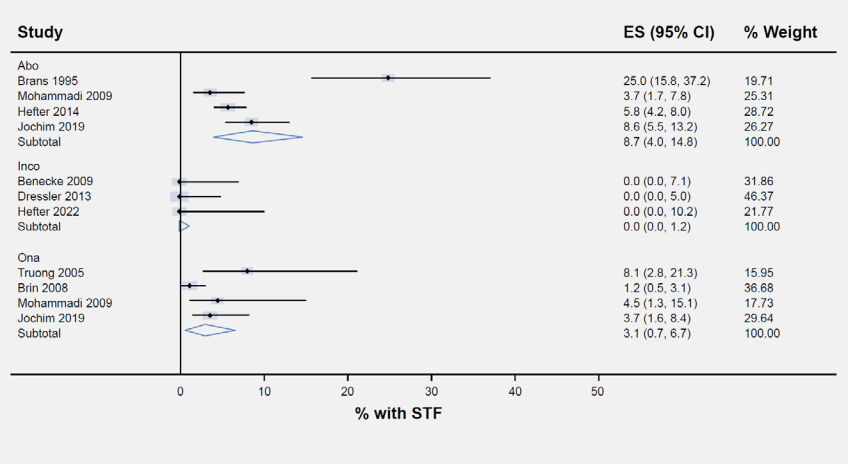
Abbreviations: Abo, abobotulinumtoxinA; BoNT, botulinum neurotoxin; CP, cerebral palsy; inco, incobotulinumtoxinA; MAS, Modified Ashworth Score; MHDA, mouse hemidiaphragm assay; MPA, mouse protection assay, NAbs, neutralizing antibodies; ona, onabotulinumtoxinA; PSS, post-stroke spasticity; TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale.

[a]Studies reporting multiple indications appear more than once.

[b]For studies that used more than one method to test for NAbs, the most sensitive one is listed even if only used for confirmation of positivity by a less sensitive test.

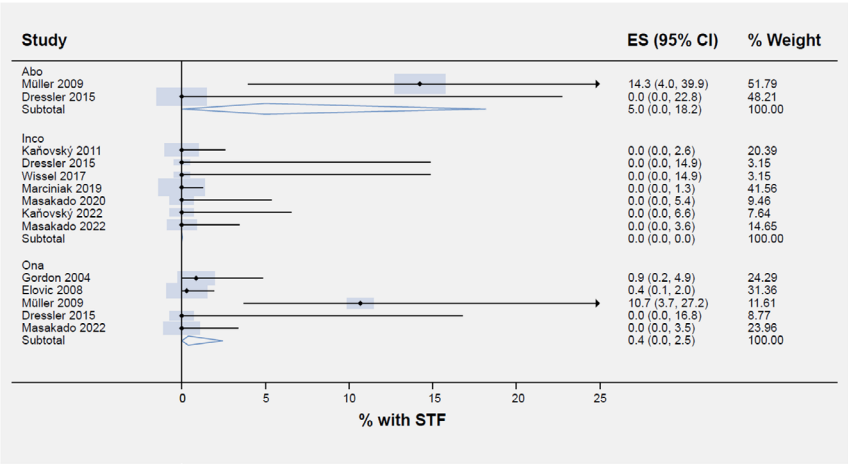
[c]Some patients may have previously received another toxin formulation, but this number is not reported in the publication.

A.



Pairwise comparisons			
	Abo vs. Inco	Abo vs. Ona	Inco vs. Ona
P-value	**<0.001**	0.08	**0.03**

B.



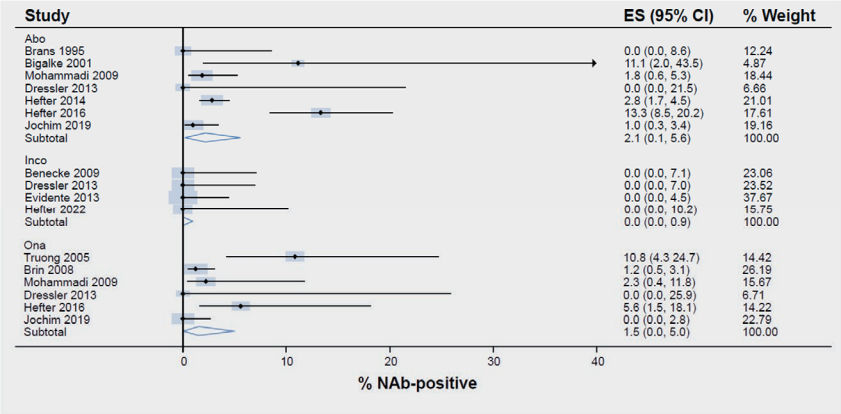
Pairwise comparisons			
	Abo vs. Inco	Abo vs. Ona	Inco vs. Ona
P-value	**0.01**	0.10	0.11

FIGURE 1 | Proportions of patients with secondary treatment failure after botulinum neurotoxin A (BoNT-A) treatment (neutralizing antibody status uncertain for some patients). (A) Cervical dystonia; (B) spasticity. Bold font indicates statistical significance ($p \leq 0.05$). Abo, abobotulinumtoxinA; CI, confidence interval; ES, effect size; Inco, incobotulinumtoxinA; Ona, onabotulinumtoxinA; STF, secondary treatment failure.

significantly higher versus incobotulinumtoxinA ($p = 0.009$) and approached significance for onabotulinumtoxinA versus incobotulinumtoxinA ($p = 0.08$, Figure 3B); the difference between onabotulinumtoxinA versus incobotulinumtoxinA reached significance when all antibody testing was considered (Figure 3A).

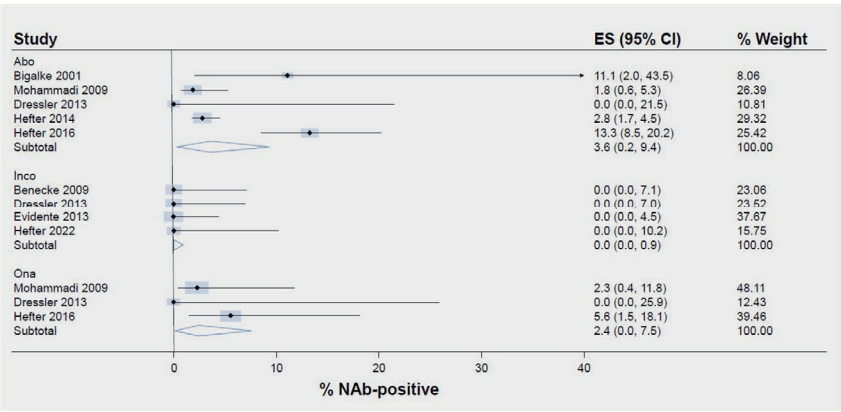
Regarding blepharospasm, fewer studies were identified assessing NAb positivity in this indication (three studies; one per toxin formulation) than for CD (12 studies) or spasticity (15 studies). Only patients receiving abobotulinumtoxinA as first-line treatment were found to have a proportion that were NAb positive (12.5%), whereas no patients receiving incobotulinumtoxinA or onabotulinumtoxinA were NAb positive. This difference was significant for abobotulinumtoxinA versus onabotulinumtoxinA ($p = 0.04$) and approached significance for abobotulinumtoxinA versus incobotulinumtoxinA ($p = 0.07$) (Figures S5 and S6).

A.



	Pairwise comparisons		
	Abo vs. Inco	Abo vs. Ona	Inco vs. Ona
P-value	**0.02**	0.72	0.07

B.



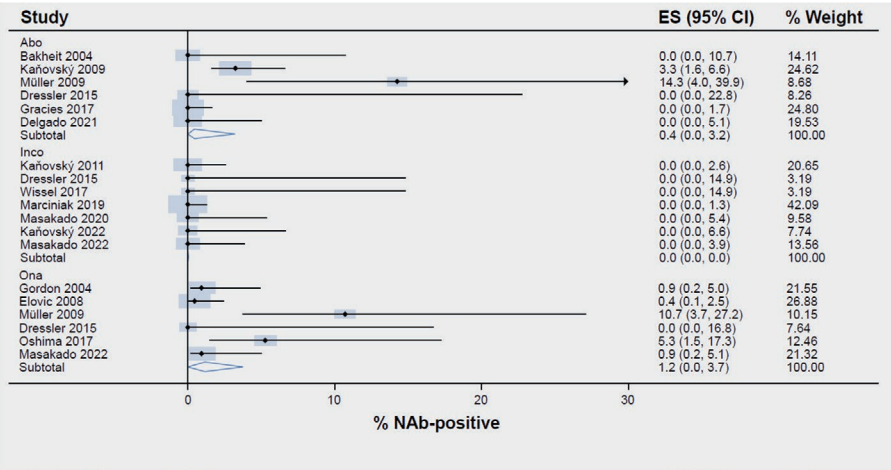
	Pairwise comparisons		
	Abo vs. Inco	Abo vs. Ona	Inco vs. Ona
P-value	**0.01**	0.77	**0.02**

FIGURE 2 | Forest plots presenting proportions of patients with cervical dystonia and neutralizing antibodies (NAbs) by first-line botulinum neurotoxin (BoNT) formulation. (A) All studies; (B) studies using mouse hemidiaphragm assay testing. Bold font indicates statistical significance ($p \leq 0.05$). Abo, abobotulinumtoxinA; CI, confidence interval; ES, effect size; Inco, incobotulinumtoxinA; Ona, onabotulinumtoxinA.

3.6 | Dose Findings

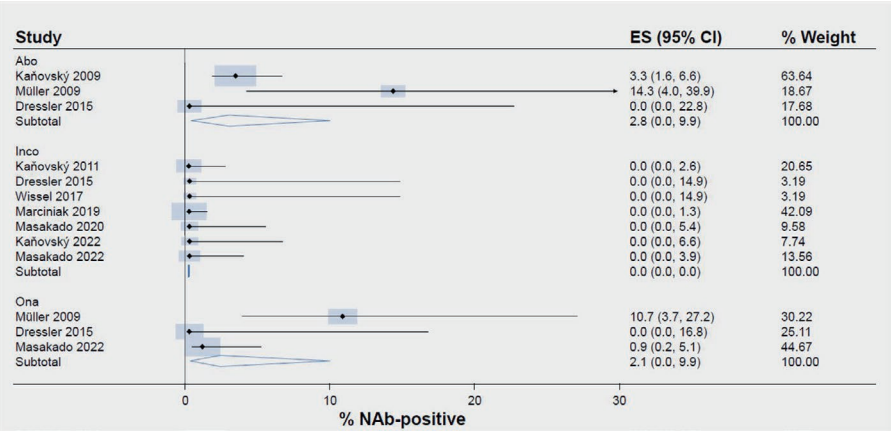
To investigate the influence of the treatment dose, a tertile split was performed for the different formulations dividing the NAb-positive patients into an upper-, a middle-, and a lower-dose tertile group. This tertile mean dose per injection cycle stratification is presented in Figures 4 and S7 for abobotulinumtoxinA and onabotulinumtoxinA; incobotulinumtoxinA was not included in these analyses as there were no NAb-positive patients treated with incobotulinumtoxinA. The proportion of patients that were NAb positive after abobotulinumtoxinA was numerically higher for the middle-dose (434–722 units, 3.2% positive) and upper-dose (> 722 units, 1.4% positive) tertiles than for the lower-dose tertile (< 434 units, 0.2% positive); however, the between-group *p*-value was not significant (Figure 4A). A similar pattern was observed with onabotulinumtoxinA, where the lower, middle, and upper tertiles were < 186 units, 0.1% positive; 186–239 units, 1.1% positive; and > 239 units, 2.0% positive, respectively (Figure 4B). No patients treated exclusively with incobotulinumtoxinA had persistent NAb positivity at any dose.

A.



	Pairwise comparisons		
	Abo vs. Inco	Abo vs. Ona	Inco vs. Ona
P-value	0.11	0.71	**0.01**

B.



	Pairwise comparisons		
	Abo vs. Inco	Abo vs. Ona	Inco vs. Ona
P-value	**0.009**	0.74	0.08

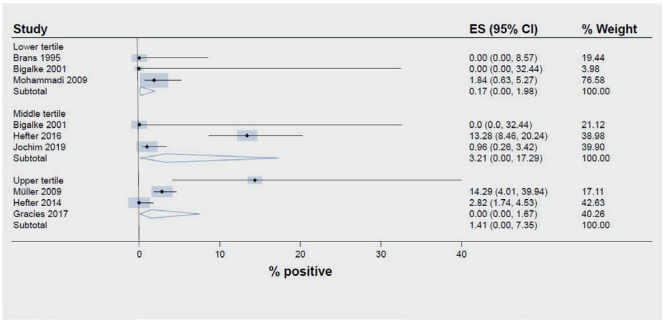
FIGURE 3 | Forest plots presenting proportions of patients with spasticity and neutralizing antibodies by first-line botulinum neurotoxin (BoNT) formulation. (A) All studies; (B) Studies using mouse hemidiaphragm assay testing. Bold font indicates statistical significance ($p \leq 0.05$). Abo, abobotulinumtoxinA; CI, confidence interval; ES, effect size; Inco, incobotulinumtoxinA; NAb, neutralizing antibody; Ona, onabotulinumtoxinA.

4 | Discussion

The reported frequencies of NAbs following BoNT injections are generally higher among conditions that are usually treated with higher BoNT doses, such as urological conditions, spasticity, and especially dystonia [51]. In some studies, NAb formation was reported to be lower with both abobotulinumtoxinA and onabotulinumtoxinA injections for glabellar lines than for CD [17, 52]. Regarding injection site, injections into anatomical regions rich in lymph nodes, such as the neck, appear more likely to produce an immune response than injections into other locations in some studies [14, 53] but not in others [11]. In addition, injection guidance techniques improve precision but are not always used or available, especially ultrasound guidance [54]. This may also contribute to the variance in STF and NAb incidence reported between studies.

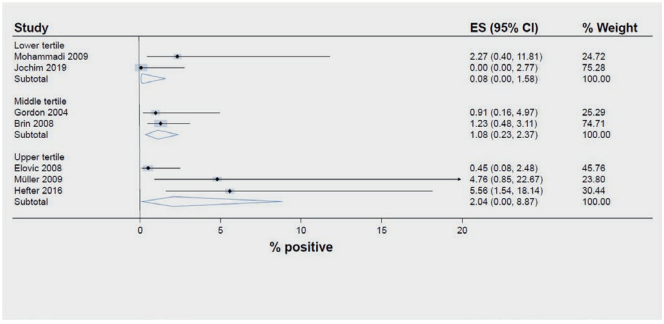
In this meta-analysis, the incobotulinumtoxinA group had zero patients with persistent NAbs (despite all identified studies of incobotulinumtoxinA using the sensitive MHDA,

A.



Dose stratification	
Lower tertile	<434 units
Middle tertile	434–722 units
Upper tertile	>722 units
Between-group P-value	0.72

B.



Dose stratification	
Lower tertile	<186 units
Middle tertile	186–239 units
Upper tertile	>239 units
Between-group P-value	0.41

FIGURE 4 | Proportions of patients with neutralizing antibodies (all indications, all testing methods) stratified by tertile mean dose per treatment cycle. (A) Abo; (B) Ona. Abo, abobotulinumtoxinA; CI, confidence interval; ES, effect size; Ona, onabotulinumtoxinA.

increasing the chance of finding NAb-positive patients) or STF, regardless of indication. Patients with CD treated with first-line abobotulinumtoxinA had relatively higher proportions with NAbs (≤4%) and STF (≤9%). Rahman et al. [55] conducted a meta-analysis of 43 studies involving 8833 patients over the period 2000–2020 and reported an overall incidence of NAbs after BoNT-A treatment of 1.8% (all indications). Our study indicated NAb rates after first-line abobotulinumtoxinA as high as 5% and 2% after first-line abobotulinumtoxinA in patients treated for CD or spasticity. There were no persistently NAb-positive patients treated exclusively with first-line incobotulinumtoxinA, regardless of dose. Our meta-analysis provides a breakdown of BoNT-A formulations per indication, whereas Rahman et al. [55] only provided a BoNT-A formulation breakdown for all indications combined. We also provide subgroup results for patients tested with the MHDA assay to show data for the most sensitive NAb assay available. Furthermore, we excluded studies that specifically recruited patients with STF, whereas Rahman et al. [55] did not. Conversely, the Rahman et al. [55] review omitted some important publications that were published at the time of their literature search and should have been eligible for inclusion, including the studies by Brans et al. [24], Hefter et al. [26, 31], Jochim et al. [30], Benecke et al. [29], Dressler et al. [27, 47], and Marciniak et al. [44] To our knowledge, our meta-analysis is the first to explore STF and immunogenicity at the level of BoNT-A formulation and indication, as well as considering immunogenicity data by the accuracy of testing used.

4.1 | Clinical Non-Responsiveness

The proportions of patients with STF in this pooled analysis appeared to be higher in patients with CD than in those with spasticity, suggesting a higher risk of STF for patients with CD treated with BoNT-A than for patients with other conditions. This may result from the more complex injection techniques required for the BoNT treatment of CD, as some muscles are difficult to access, which can increase the risk of imprecise injection [56]. Furthermore, it is likely to be at least partially linked to

NAb development, which has been reported to occur more frequently in CD [14]. As with immunogenicity, this finding could be due to the greater number of published studies specifically investigating STF in CD than in spasticity. We were unable to perform an analysis specifically focusing on patients who were both NAb positive and developed STF because these outcomes were very inconsistently investigated and reported in the included studies; in most cases, numbers of patients with NAbs were reported independently from patients with STF within a single study, without specifying how many patients experienced both phenomena.

Some investigators have reported that NAb formation is associated with STF. For example, Hefter et al. [57] found that the presence of NAbs was clinically relevant in CD, leading to a significantly worse head position, therapy with significantly higher BoNT-A doses, and a correlation between the pain sub-score and antibody titres. Similarly, Lee et al. [58] reported that NAb-positive patients were treated with higher doses and perceived a less effective reduction of subjective symptoms compared with NAb-negative patients. Management of BoNT-A therapy was strongly influenced by the risk of inducing NAbs, and the risk of NAb formation was underestimated because many studies have too short a duration of treatment. This means studies are reporting the incidence of NAbs, not total prevalence [57].

Other authors question whether STF can only be due to NAb formation. A systematic and meta-analytic review of the frequency of NAbs among patients treated with BoNT therapy for any clinical indication found that, among all patients ($n = 8525$), the frequency of NAbs was 5.9% for spasticity (0.7% for responders and 75.9% for non-responders). About half of patients with STF did not have NAbs, and the prevalence of NAbs was lower among clinically responding patients. In patients with secondary non-responsiveness, the rate of NAbs was 56.7% with abobotulinumtoxinA, 0% with incobotulinumtoxinA, and 32.5% with onabotulinumtoxinA [51].

Bellows and Jankovic [9] suggested the most notable uncertainty was the exact relationship between NAbs and STF. Many patients continue to benefit from BoNT-A therapy despite the presence of NAbs, and STF is frequently attributed to other causes. This uncertainty is partly due to a lack of quantitative, specific, and sensitive assays for NAbs [9, 59]; there does not seem to be a particular threshold for NAb titer above which clinical resistance occurs [8]. Self-perceived symptom reduction, which may be linked to adherence to BoNT, has been shown to vary by indication, with self-perceived efficacy higher with CD and blepharospasm than with spasticity [58]. This could explain why immunogenicity is lower in spasticity than CD despite higher doses (poor adherence and therefore lower longitudinal exposure to BoNT) and also why NAb titres may not correspond to clinical response. For example, results in clinical practice are conflicting. Sometimes a patient has an excellent clinical response but also has a NAb titer that can be detected in the MHDA. Other times, the clinical outcome may be unsatisfactory but the immunogenicity test is negative or close to threshold. For example, about 50% of patients with clinical partial STF have a negative MHDA test [51, 60]. These findings may reflect levels of NAbs that are below the detection limit of the MHDA and MLA assays [57], and this implies that NAb positivity is not the only factor driving STF. Another possibility could be that the MHDA is not sensitive enough to detect NAbs in these patients, and NAbs may be present at titres beyond the sensitivity of the assay in the patients [20]. Furthermore, differing sensitivities of antibody assays may explain at least part of the imperfect correlation between NAb positivity and STF [9]. Other possible reasons for nonresponse include insufficient dose, inappropriate muscle selection, improper injection technique or targeting, natural progression of underlying disease, difficulty in treating condition, poor test sensitivity to detect NAbs, and changes in patient expectations [13, 51, 55, 57].

In patients with partial STF, increasing the dose of BoNT-A can improve efficacy, but the required increase may be as high as four times the normal dose [9], reaching the maximum dose specified in the product label and potentially inducing complete STF due to the increased risk of NAb development at higher doses with some BoNT-A formulations. An alternative approach is to discontinue treatment with BoNT-A until NAb levels have dropped and then resume treatment with a BoNT-A formulation that lacks complexing proteins, such as incobotulinumtoxinA [9]. The differing findings and opinions on the clinical relevance of NAb positivity in the literature and the lack of access to routine sensitive NAb testing outside of the clinical trial environment limit the implication of immunogenicity findings for clinical practice. On the other hand, secondary treatment failure after BoNT-A treatment is an important clinical problem that physicians may encounter, so they need to be aware of BoNT-A resistance and the associated risks. Findings of the present review highlight that, of the three BoNT-A formulations that have been in routine clinical use for multiple indications for more than 15 years, incobotulinumtoxinA is the only formulation with no reported case of immunogenic STF. Consequently, the incidence of STF in patients treated with incobotulinumtoxinA in CD and spasticity is significantly lower than for patients treated with abobotulinumtoxinA or onabotulinumtoxinA (Figure 1).

4.2 | Immunogenicity

Our pooled analysis shows NAb rates in patients treated for CD as low as 0% (in patients treated exclusively with incobotulinumtoxinA) and as high as 7% (in patients treated with first-line abobotulinumtoxinA). By comparison, the meta-analysis by Rahman et al. [55] reported a proportion of patients with dystonia (any form) who were NAb positive as 7.4% (all BoNT-A formulations pooled). The Rahman et al. [55] meta-analysis found no difference between the incidence of NAb-positive patients for onabotulinumtoxinA versus incobotulinumtoxinA when all indications were pooled; conversely, we found a significantly higher proportion of NAb-positive patients with CD who had received onabotulinumtoxinA or abobotulinumtoxinA when compared with incobotulinumtoxinA (Figure 2). Transient NAb positivity on serial MHDA has been detected incidentally in two patients with CD treated exclusively with incobotulinumtoxinA for more than 3 years. However, both patients showed negative NAb titres at follow-up investigation and stable treatment response thereafter for more than 9 years [14].

Regarding spasticity, our meta-analysis shows NAb rates as low as 0% (in patients treated exclusively with incobotulinumtoxinA)

and as high as 2.8% (in patients treated with first-line abobotulinumtoxinA), whereas Rahman et al. [55] reported a proportion of patients who were NAb positive of 6.7% (all BoNT-A formulations pooled). As with CD, there was a significantly lower proportion of NAb-positive patients with incobotulinumtoxinA than with abobotulinumtoxinA or onabotulinumtoxinA in our meta-analysis (Figure 3). Albrecht et al. [11] noted that the recent trend of using high doses of BoNT-A per session for the treatment of spasticity may come at the cost of higher rates of NAb positivity. Our data and that of Albrecht et al. [11] suggest that BoNT-A formulation may have a clinically significant influence on the risk of developing NAbs and that incobotulinumtoxinA treatment may be associated with lower rates of NAbs, although this needs to be corroborated in larger cohorts with more homogeneous follow-up times than studies published to date.

In the present study, the proportion of patients treated for blepharospasm with onabotulinumtoxinA who become NAb positive was also numerically lower (0%) than for patients with spasticity or CD. Conversely, a relatively high proportion of patients treated with abobotulinumtoxinA for blepharospasm (13%) was found (Figure S2). This finding is likely to be a limitation of the data in blepharospasm, with only individual studies per toxin formulation identified and small numbers of patients ($n = 8$ for the study of abobotulinumtoxinA in blepharospasm [32]). In the meta-analysis by Rahman et al. [55] and the multiple indication study by Albrecht et al. [11], the incidence and prevalence (respectively) of NAb-positive patients was higher for CD and spasticity than for blepharospasm. This difference was attributed to the much lower dosing of BoNT for blepharospasm than for spasticity or CD [11]. No patients with blepharospasm treated with incobotulinumtoxinA became NAb positive.

4.3 | Dose Per Injection

When NAb positivity by dose of toxin was investigated, there appeared to be a non-significant trend for a numerically higher incidence of patients with NAbs at medium and high doses of abobotulinumtoxinA and onabotulinumtoxinA compared with the low-dose tertiles. The proportion of patients treated with incobotulinumtoxinA who were NAb positive was 0%, regardless of dose or indication.

High BoNT-A dose per treatment (> 675 unified dose units) was a risk factor for NAb-complete STF in a study of multiple BoNT-A indications by Walter et al. [14] Similarly, Albrecht et al. [11] found a higher risk in patients receiving mean doses of > 350 unified dose units, with the highest risk in those receiving > 700 unified dose units; single dose per session was the second most influential factor for NAb development [11].

Doses of BoNT given for spasticity are typically higher than for CD, so it is interesting to note that we identified greater proportions of NAb-positive patients in studies of CD than in those with spasticity, a finding echoed by an earlier systematic review by Mathevon et al. [61] This may be due to the follow-up times in the respective indications, which were generally shorter in the spasticity studies. Still, a potentially stronger systemic immune response to injection of the neck muscles has been discussed [14]. However, we cannot exclude that this finding is due to the greater number of published studies in CD than in spasticity specifically investigating immunogenicity after treatment with BoNT.

4.4 | Impact on Clinical Practice

Some patients, especially children and adolescents, may require long-term treatment with BoNT-A [9]. An emerging body of evidence and current guidelines emphasize the importance of approaching BoNT-A treatment of chronic conditions with protocols to minimize the immune response and maximize patient responsiveness [10]. Immunogenicity should therefore be a key factor in the choice of BoNT-A therapy; where efficacy and safety are comparable, a BoNT-A formulation that is potentially less likely to cause immunogenicity should be considered as a first-line therapy [20, 61]. Thus, initiating treatment with a BoNT-A formulation with a lower potential immunogenicity may reduce the risk of NAb production and future treatment failure, especially if higher doses of BoNT-A are applied [14, 62, 63]. Choice of BoNT-A formulation is especially important since sensitive, affordable, standardized tests are not currently available to allow routine monitoring of NAbs during BoNT-A treatment [9]. There is a need for better, affordable, and standardized NAb testing to allow routine monitoring of NAb levels during BoNT-A treatment, to better monitor patients, and to help identify reasons for partial or full non-response to BoNT therapy.

Exposure to BoNT-A has drastically increased in recent years, especially for esthetic purposes and in younger age groups. As a consequence, cumulative lifetime exposure and therefore the risk of STF and NAb positivity increases with time in many people, even before receiving an initial dose of BoNT-A for therapeutic purposes. There appears to be a cumulative risk in developing NAbs against abobotulinumtoxinA or onabotulinumtoxinA and immunogenic STF, but not against incobotulinumtoxinA.

It should be stressed that the occurrence of NAbs does not inevitably lead to STF. In many patients, an adequate clinical effect can be restored by dose escalation or by optimizing injection sites. Nevertheless, when standard fixed doses are applied, treatment response is attenuated in the presence of NAbs, and several large real-world cohorts have demonstrated poorer outcomes in NAb positive compared with NAb negative patients [14, 58]. In previous studies, it has also been shown that changing from one BoNT A formulation to another increases immunogenic risk [11, 14]. This seems to apply especially for switching between onabotulinumtoxinA and other BoNT-A formulations, except for switching from onabotulinumtoxinA to incobotulinumtoxinA [14]. There is still some uncertainty around whether patients switch as a result of immunogenicity leading to STF, or whether the switch itself triggers immunogenicity. For example, Walter et al. found that switching from onabotulinumtoxinA to abobotulinumtoxinA or rimabotulinumtoxinB but not incobotulinumtoxinA was a risk factor for developing immunogenic STF in patients who still experienced a therapeutic effect at the time of switch [14]. In another study, however, 45% of patients with STF were found to have neutralizing antibodies, the majority of whom had not switched BoNT formulation (onabotulimtoxinA or abobotulinmumtoxinA) [60]. Clinicians should therefore reserve toxin switching for clear practical or pharmacological

reasons, for example, switching to a less immunogenic formulation if NAbs are suspected in order to avoid further increasing NAb titres.

When data from patients initially treated with abobotulinumtoxinA or onabotulinumtoxinA (multiple indications) and switched to another BoNT-A were pooled, the risk of developing NAbs reached 70% within 20 years of initiating first-line BoNT-A treatment [63]. However, patients with STF and/or NAbs after treatment with abobotulinumtoxinA or onabotulinumtoxinA responded, and their NAb concentrations decreased when their BoNT was switched to incobotulinumtoxinA [63–66]. The potential cumulative exposure (also known as lifetime exposure) to different BoNT-A formulations for different indications is something that clinicians need to be increasingly aware of. Therefore, where the efficacy and safety of other BoNT formulations are comparable, the authors recommend the use of incobotulinumtoxinA to avoid and/or manage the development of STF and NAbs.

There are a number of limitations with this meta-analysis. As immunogenicity was often not the primary focus of the identified publications, methodology and reporting of data was inconsistent between studies. Furthermore, STF definitions varied between studies or were not defined, limiting the comparability of each study. The effect of treatment duration was not assessed as the majority of publications did not report these data at the level of BoNT-A formulation. Patient populations also varied between studies in terms of age, duration of follow-up, and (in the case of spasticity) etiology. Finally, testing methods for NAbs and definitions for STF varied between studies.

5 | Conclusion

The risk of developing STF and NAbs after treatment with BoNT-A appears to vary with indication as well as formulation of BoNT-A received. In addition, there may be a cumulative risk over time that is not immediately apparent from the short-term clinical trials included in this review. Our meta-analysis found that incobotulinumtoxinA was associated with a significantly lower risk of developing immunogenic STF or persistent NAbs compared with onabotulinumtoxinA or abobotulinumtoxinA in patients with CD or spasticity. Consequently, when alternative formulations of BoNT have comparable efficacy and safety, the use of incobotulinumtoxinA may be recommended to avoid the development of STF and NAb formation, particularly for patients who require higher doses and repeated treatments. In fact, no patients treated exclusively with incobotulinumtoxinA developed immunogenic STF or persistent NAbs that were identified in our meta-analysis, supporting this choice of BoNT formulation.

A consistent approach is needed for STF definitions and NAb testing approaches in future studies of BoNT-A, as are additional head-to-head studies, to more precisely define the risk of developing STF and NAbs in different clinical scenarios with different BoNT preparations. Furthermore, prospective studies of longer observation than conducted previously are required in order to better assess the cumulative risk of developing STF and NAbs. It must be kept in mind that it is extremely difficult to keep the technical and biological conditions constant for the performance of long-term serial MHDA measurements. Better recognition of the occurrence and minimization of the risk of STF and NAbs will benefit patients in terms of improved, consistent outcomes from BoNT-A therapy, many of whom require life-long treatment.

Author Contributions

Uwe Walter: conceptualization, investigation, methodology, validation, visualization, writing – review and editing, writing – original draft, supervision. **Phillipp Albrecht:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, supervision. **Warner Carr:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, supervision. **Harald Hefter:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, supervision.

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Conflicts of Interest

U.W. has received speaker honoraria and travel reimbursement from Amarin, Bristol-Myers Squibb, Daiichi Sankyo, Ipsen Pharma, Merz Therapeutics, and Allergan/AbbVie, and an unrestricted research grant from Merz Therapeutics. He has received compensation from Thieme as joint Editor-in-Chief of the *European Journal of Ultrasound*, outside the submitted work. P.A. has received speaker honoraria, consulting honoraria, and travel reimbursement from Allergan, AbbVie, Biogen, Bristol-Myers Squibb, Celgene, Ipsen, Janssen-Cilag, Johnson & Johnson, Lilly, Merck, Merz Therapeutics, Novartis, Pfizer, Roche, Sanofi, and Teva. He has received research grants from Deutsche Forschungs Gesellschaft (DFG), EFRE-NRW, Biogen, Bristol-Myers Squibb, Celgene, Ipsen, Merck, Merz Therapeutics, Novartis, and Roche. W.C. has served as a paid consultant for Merz Therapeutics GmbH and Merz Pharmaceuticals LLC. H.H. has received speaker honoraria, consulting honoraria, and travel reimbursement from Allergan, Ipsen, Merz Therapeutics, and Teva. He has also received research grants from the Deutsche Forschungsgemeinschaft (DFG) and three grants from the European Union (EFRE-NRW) and two restricted grants from the German Wilson disease patient organization.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.