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Safety and efficacy of liquid nitrogen spray cryotherapy in Barrett's neoplasia – a comprehensive review and meta-analysis

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ABSTRACT

Background and study aims Barrett's esophagus (BE) is a precursor condition to esophageal adenocarcinoma (EAC), resulting in transformation of the squamous epithelium of distal esophagus to columnar-lined epithelium with intestinal metaplasia (IM). Liquid nitrogen spray cryotherapy (LNSC) is a non-contact method of BE eradication and has been used both as primary and salvage therapy. We conducted a systematic review and meta-analysis to assess the safety and efficacy of LNSC.

Methods We searched multiple databases from inception through December 2021 to identify studies on use of LNSC for Barrett's neoplasia. Pooled estimates were calculated using random-effects model and results were expressed in terms of pooled proportions with relevant 95 % confidence intervals (CIs) of complete eradication (CE) of dysplasia(D), high grade dysplasia (HGD) and IM.

Results Fourteen studies with 707 patients were included in our final analysis. Overall pooled rates of CE-D, CE-HGD and CE-IM were 80.8 % (CI 77.4–83.8; I^2 62), 90.3 % (CI 85.2–93.7; I^2 33) and 55.8 % (CI 51.7–59.8; I^2 73) with fol-

low up ranging from 4.25 months to 69.7 months. In patients with follow up beyond 24 months, the rates of CE-D and CE-IM were 83.6 % (CI 77.6–88.2; I^2 60) and 54.7 % (CI 47.6–61.6; I^2 81). Among LNSC naïve patients with prior history of endoscopic resection, the rates were 79.9 % (CI 73.3–85.2; I^2 50) and 67.1 % (CI 59.5–73.8; I^2 0). Pooled rate of therapeutic failures, defined as lack of response to LNSC therapy, was 23.6 % (CI 19.4–28.3; I^2 73). Post LNSC strictures and perforation pooled rates were 4 % and 0.8 %, respectively, which are similar to those previously reported for RFA.

Introduction

Barrett's esophagus (BE) is a premalignant condition where there is transformation of the squamous epithelium of the distal esophagus to columnar-lined epithelium with intestinal metaplasia (IM). It classically develops due to chronic inflammation from gastroesophageal reflux (GERD), and while the annual malignant conversion risk of IM is only 0.3 %, [1] low-grade dysplasia (LGD) carries a 0.2 % to 1.5 %, and high-grade dysplasia (HGD) an annual risk of 5 % to 8 % of progression to esophageal adenocarcinoma (EAC), respectively [1–4]. Therefore, timely diagnosis and management of BE remains paramount. Management of non-dysplastic BE includes chemoprevention via proton-pump inhibitor therapy and endoscopic surveillance [5]. However, given the risk of progression to EAC, patients with BE harboring dysplasia are recommended to undergo endoscopic eradication therapy (EET), with the intent to achieve complete eradication of intestinal metaplasia (CE-IM). This has been shown to reduce progression to HGD or EA when, compared to surveillance alone in patients with LGD [6]. In patients with HGD, EET has shown to be efficacious and to have a favorable side effect profile compared to esophagectomy [7].

Endoscopic resection techniques such as endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) are indicated for treatment of superficial esophageal cancer and BE-associated neoplasia (high-grade dysplasia (HGD) and intramucosal carcinoma (IMC) [8, 9], however these are often not sufficient for achieving CE-IM. Endoscopic ablation using photochemical, freezing, or thermal injury aims to eliminate BE by inducing superficial necrosis of the metaplastic tissue, which can effectively eliminate dysplastic potential and allow for re-epithelialization with neo-squamous epithelium [10]. Radiofrequency ablation (RFA) has been the most widely used and studied ablative technology in this regard and is considered the primary therapy for dysplastic BE [11].

Cryotherapy is a non-contact method of BE eradication which involves application of cryogen resulting in tissue destruction. This can be performed with either spray cryotherapy using liquid nitrogen (LNSC) [12] or carbon dioxide gas [13] or cryoballoon ablation (CBA) using nitrous oxide gas [14]. The major difference between liquid nitrogen and carbon dioxide based modalities is the temperature of cryogen, i. e., –85 °C for carbon dioxide and –76 °C to –158 °C for liquid nitrogen [15]. Data regarding use of LNSC as primary as well as salvage therapy [16] for dysplastic BE continues to emerge. Prior meta-analysis performed are limited by inclusion of non-liquid nitrogen-based cryotherapy modalities [17] and small patient cohorts [18, 19]. We conducted a comprehensive review and meta-analysis to assess the safety and efficacy of cryotherapy using LNSC in both ablation naïve and experienced patients.

Methods

Search strategy

The relevant medical literature was searched by a medical librarian for studies reporting on the outcomes of LNSC in Barrett's Esophagus. The search strategy was created using a combination of keywords and standardized index terms. A systematic and detailed search was run in December 2021 in Ovid EBM Reviews, ClinicalTrials.gov, Ovid Embase (1974 +), Ovid Medline (1946 + including epub ahead of print, in-process & other non-indexed citations), Scopus (1970 +) and Web of Science (1975 +). Literature search was performed to include studies published in all languages, and in the case of non-English studies, electronic language translation service was used to convert the text to English. All citations were downloaded onto EndNote software. The review was not registered, and a protocol was not prepared.

The full search strategy is available in **Supplementary Appendix 1**. For observational studies, the MOOSE (Meta-analyses Of Observational Studies in Epidemiology) Checklist was followed [20] and is provided as **Supplementary Appendix 2**. PRISMA Flowchart for study selection is provided as **Supplementary Fig. 1**. Reference lists of evaluated studies were examined to identify other studies of interest.

Study selection

In this meta-analysis, we only included studies where outcomes of liquid nitrogen spray cryotherapy (LNSC) were reported. We excluded studies assessing cryotherapy using carbon dioxide and nitrous-oxide balloon-based ablation system. We included studies where LNSC was performed using both the G2 system (from 2007 to 2012, CSA Medical, Lexington, Massachusetts, United States) and truFreeze device (from 2013 to present, CSA Medical Inc., Baltimore, MA). Studies included randomized controlled trials, cohort, and case-control studies that reported outcomes of interest. Studies were included irrespective of whether they were performed in the inpatient or outpatient setting, follow-up time, and country of origin as long as they provided the appropriate data needed for the analysis.

Our exclusion criteria were as follows: (1) studies reporting outcomes from non-liquid nitrogen-based cryotherapy modalities; (2) studies reporting outcomes of other ablation techniques such as RFA, argon plasma coagulation (APC) and/or

photodynamic therapy (PDT), unless reported as a comparative group in studies on LNSC; (3) single patient case reports and case series; (4) studies with sample size < 10 patients; (5) studies published only as conference abstracts; and (6) studies performed in the pediatric population (Age < 18 years). In cases of multiple publications from a single research group reporting on the same patient cohort and/or overlapping cohorts, data from the most recent and/or most appropriate comprehensive report were retained. The retained studies were determined based on the publication timing (most recent) and/or the sample size of the study (largest). In situations where a consensus could not be reached overlapping studies were included in the final analysis and any potential effects were assessed by sensitivity analysis of the pooled outcomes by leaving out one study at a time.

Data abstraction and quality assessment

Data on study-related outcomes from the individual studies were abstracted independently onto a standardized form by at least two authors (SC, SRK, JP). Authors (DR, HG, MA) cross-verified the collected data for possible errors and two authors (SC, SRK) performed the quality scoring independently.

Outcomes assessed

We calculated pooled rates for the following outcomes:

Efficacy outcomes

1. Complete eradication of dysplasia (CE-D) – defined as endoscopic and histological remission of all dysplasia.
2. Complete eradication of high-grade dysplasia (CE-HGD) – defined as eradication of all high-grade dysplasia but with either persistent LGD or persistent non-dysplastic intestinal metaplasia.
3. Complete eradication of intestinal metaplasia (CE-IM) – defined as no visible endoscopic evidence of BE and histological remission of intestinal metaplasia.
4. Recurrence of dysplasia (RE-D) and intestinal metaplasia (RE-IM) – defined as histologic evidence of intestinal metaplasia, dysplasia, or neoplasia on endoscopic biopsy during the surveillance period, after achieving CE-IM and/or CE-D
5. Failure (F) – defined as lack of response to therapy, demonstrated by persistence of the previously diagnosed intestinal metaplasia, dysplasia, or cancer, or progression to worsening dysplasia or cancer.

We further sub-grouped our pooled results of CE-D, CE-HGD and CE-IM into the following categories:

a) LNSC-naïve patients with prior history of endoscopic resection, i. e. EMR and/or ESD
b) Studies with short term (up to 24 months) and long term (> 24 months) follow up.

Safety outcomes

1. Pooled incidence of post therapy strictures
2. Pooled incidence of post therapy perforation
3. Pooled incidence of post therapy pain

Statistical analysis

We used meta-analysis techniques to calculate the pooled estimates in each case following the methods suggested by DerSimonian and Laird using the random-effects model and results were expressed in terms of pooled proportion (PP) along with relevant 95 % confidence intervals (Cis) [21]. When the incidence of an outcome was zero in a study, a continuity correction of 0.5 was added to the number of incident cases before statistical analysis [22]. We performed subgroup analysis to compare outcomes in ablation naïve patients (without history of prior ablation therapy) and LNSC naïve patients with history of prior EMR and/or ESD. $P < 0.05$ was used a-priori to define significance between the groups compared and considered as descriptive only as they were uncorrected for multiple testing.

We assessed heterogeneity between study-specific estimates by using Cochran Q statistical test for heterogeneity, 95% confidence interval (CI) and the I^2 statistics. [22–24] In this, values of < 30 %, 30 % to 60 %, 61 % to 75 %, and > 75 % were suggestive of low, moderate, substantial, and considerable heterogeneity, respectively. We assessed publication bias, qualitatively, by visual inspection of funnel plot and quantitatively, by the Egger test [25]. When publication bias was present, further statistics using the fail-Safe N test and Duval and Tweedie's 'Trim and Fill' test was used to ascertain the impact of the bias [26]. All analyses were performed using Rstudio software, version

Results

Search results and population characteristics

All search results were exported to Endnote where 596 obvious duplicates were removed leaving 442 citations. A total of 14 studies (13 retrospective [16, 27–39] and one prospective cohort [40]) with a total of 707 patients were included in the final analysis. A schematic diagram demonstrating our study selection is illustrated in **Supplementary Fig. 1**. The patient population was LNSC-naïve with history of EMR/ESD in five studies [32, 34, 37–39] and treatment-experienced in nine studies.

Prior ablation therapies included APC in seven patients, PDT and/or RFA in 140 patients, and EMR and/or ESD in 243 patients. Further details of number of LNSC cycles used, median number of sessions, prior ablation therapies, BE length along with dysplasia subtype are described in ▶ **Table 1**, ▶ **Table 2**, ▶ **Table 3**.

Characteristics and quality of included studies

All the included studies were published as full-length manuscripts. Five studies were multicenter whereas 9 studies were single center experiences. Overall mean follow-up time ranged from 4.25 months to 69.7 months. Based on the New-Castle Ottawa scoring system (**Supplementary Table 1**), one study was of medium quality [27] and all others were considered to be of high quality. There were no low-quality studies.

▶ **Table 1** Study details.

Study	Design, center, period	Therapy (G2 system –>2007 to 2012, truFreeze –>2014 to present)	Technique	Total patients	Ablation naïve/ LNSC naïve	No. of sessions/ patient	Age mean/ median [SD] (range)	Gender (male/ female)
Dumot, 2009	Non-randomized retrospective cohort study, September 2005 to September 2008, Single center, USA	Spray LN (CSA Medical Inc, Baltimore, Md)	3 cycles of 20-second cryospray (first half), 4 cycles of 10-second cryospray (second half)	31	Prior ablation therapies	5 (3–7) [Median]	69.7 [11]	21/10
Shaheen, 2010	Retrospective, 2007 to 2009, Multicenter, USA (University of North Carolina, University of Maryland/Greenebaum Cancer Center, Cleveland Clinic, Massachusetts General Hospital, Mayo Clinic Jacksonville,Texas Digestive Health Associates, Columbia University Medical Center, Mayo Clinic Rochester, Digestive Health Associates of Texas, Lancaster Gastroenterology Inc, Virginia Commonwealth University, Moffitt Cancer Center	CSA cryotherapy system (CSA Medical, Baltimore, Md).	2 cycles of 20 seconds or 4 cycles of 10 seconds.	98	Prior ablation/resection therapies	3.4 [Mean]	65 [10]	81/17
Greenwald, 2010	Prospective, September 2005 to November 2007, multicenter, USA (University of Maryland/Greenebaum Cancer Center, Cleveland Clinic, Walter Reed Army Medical Center, Columbia University Medical Center)	–	3 cycles-20 seconds, at least 45 seconds between freezes to allow tissue thawing, 4 cycles of 10 seconds (2007 onwards)	77	Naïve (EMR done)	4 (1–10) [Median]	69 [12.2]	57/10
Sengupta, 2015	Retrospective, single-center, 2006 to 2013, USA (Beth Israeil Deaconess, Boston, MA)	(CryoSpray Ablation System; CSA Medical, Inc, Lexington, Mass)	3 cycles of 20 seconds	16	Prior ablation/resection therapies	3 [Median]	–	–
Ghorbani 2016	Multicenter, prospective open-label registry, 2009 to 2012 (University of Maryland, Cleveland Clinic, North Shore LIJ, Syosset Hospital, University of North Carolina, Rhode Island Hospital, and Scripps Clinic)	CryoSpray Ablation System (2nd generation, CSA Medical, Baltimore, MD, USA)	2–3 cycles of 20-second freezes or 4 cycles of 10-second freezes	96	Prior ablation/resection therapies	3.3	67 (50–93)	80/16
Suchniak – Mussari, 2017	Retrospective, January 2010 to December 2014, Single center, USA (Allegheny General Hospital, Penn State Hershey Medical Center)	truFreeze spray cryotherapy system (CSA Medical Inc., Baltimore, Maryland, United States)	2 cycles of 20 s bursts	33	Prior ablation/resection therapies	2 (1–6) [Median/ Range]	66 [8.7]	21/12
Ramay, 2017	Retrospective, April 2006 to February 2012, Single center, USA (University of Maryland Medical Center)	(CryoSpray Ablation System; CSA Medical, Inc, Lexington, Mass)	3 cycles of 20 seconds, later changed to 4 cycles of 10 seconds, then 2 cycles of 20 seconds	40	LNSC Naive (EMR done)	3 (2–5) [1–12] {Median/IQR/ Range}	61.1 [8.0] (36–78)	37/3

▶ **Table 1** (Continuation)

Study	Design, center, period	Therapy (G2 system –>2007 to 2012, truFreeze –>2014 to present)	Technique	Total patients	Ablation naïve/ LNSC naïve	No. of sessions/ patient	Age mean/ median [SD] (range)	Gender (male/ female)
Trindade, 2017	Retrospective, 2008 to 2014, Multicenter, USA (Long Island Jewish Medical Center, Strong Memorial Hospital)	(Cryospray Ablation System; CSA Medical, Lexington, MA, USA).	3 cycles of 20 seconds	18	Prior ablation/resection therapies	3 [Median]	64.5	15/3
Trindade, 2018	Retrospective, Beth Israel Deaconess Medical Center in Boston, MA (2007 to 2015), Long Island Jewish Medical Center/North Shore University Hospital, New Hyde Park, NY (2013 to 2015), and University of Rochester Medical Center, Strong Memorial Hospital, Rochester, NY (2009 to 2015), Multicenter, USA.	(CSA Medical, Lexington, MA)	2 cycles 20 s each.	27	LNSC Naive (EMR done)	3 (Range 1–12) [Median]	68 (47–87) [Median]	24/3
Thota, 2018	Retrospective, 2006 to 2011, Single center, USA (Cleveland Clinic)	(Generation 2 device, CSA Medical, Baltimore, MD).	2–3 cycles of 20 s each, at least 45 s between freezes to allow tissue thawing	81	Prior ablation/resection therapies	3.0 (2.0, 5.0) [Median]	69.8 [10.7]	65/16
Spiceland, 2019	Retrospective, 2007 to 2018, USA (Medical University of South Carolina)	(truFreeze Spray Cryotherapy, Lexington, Massachusetts, United States)	–	46	Prior ablation/resection therapies	2	66 [Median]	42/4
Kaul, 2020	Retrospective, August 2008 to February 2019, Single center, USA (University of Rochester Medical Center and Strong Memorial Hospital)	(CSA Medical, Inc.; Baltimore, MD)	10–30-sec applications × 2–4 applications	57	Prior ablation/resection therapies	3	68.5	51/6
Fasullo, 2021	Retrospective, 2014 to 2020, Multicenter, USA (Virginia Commonwealth University Medical Center, Central Virginia Veteran's Afairs Medical Center)	truFreeze, Steris Medical, Mentor, OH	3 cycles 20–30 secs	62	Ablation Naive	4.2 (2.9) [CE-D (SD)], 4.8 (3.4) [CE-IM (SD)]	67.1 [12.3]	51/11
Alshelleh 2021	Retrospective study from a tertiary care center from 2015 to 2019	truFreeze, Steris Endoscopy, Mentor, OH	–	25	LNSC Naive (EMR done)	2–5 (2.8)	49–84 [65]	21/4

Abs, Abstract; NR, not reported; EMR, endoscopic mucosal resection; CE-D, complete eradication of dysplasia; CE-IM, complete eradication of intestinal metaplasia; SD, standard deviation; IQR, interquartile range.

Meta-analysis outcomes

Efficacy Outcomes

Pooled rates of CE-D, CE-HGD and CE-IM with LNSC – Across all studies, the overall pooled rates of CE-D, CE-HGD and CE-IM were 80.8% (95% CI [77.4–83.8]; I^2 62%), 90.3% (95% CI [85.2–93.7]; I^2 33%) and 55.8% (95% CI [51.7–59.8]; I^2 73%), respectively (▶ **Fig. 1**, ▶ **Fig. 2**, ▶ **Fig. 3**). Among 5 studies which included LNSC-naïve patients with prior history of endoscopic resection, overall pooled rates of CE-D and CE-IM were 79.9% (95% CI [73.3–85.2]; I^2 50%) and 67.1% (95% CI [59.5–73.8]; I^2 0%), respectively.

Subgroup analysis

In studies with mean/median follow-up time up to 24 months after LNSC, the pooled rates of CE-D, CE-HGD and CE-IM were 80.8% (95% CI [75.8–85]; I^2 71%), 92.2% (95% CI [86.5–95.6]; I^2 41%) and 56% (95% CI [49.9–62]; I^2 78%), respectively. Among the studies with follow-up time greater than 24 months

▶ **Table 2** Patient characteristics.

Study	Prior therapy					Barrett's length cm [SD] (Range)		Barrett's subtype			
	APC	PDT	RFA	EMR/ ESD	Others	Short segment	Long segment	Non-dysplastic	Low-grade (LGD)/indefinite dysplasia	High-grade dysplasia (HGD)	IMC (T1a/ T1b)
Dumot, 2009	2	3	–	4	–	–	6.1 [4.1] (1–15)	–	–	30	
Shaheen, 2010	2	6	6	22	Esophagectomy 2/98, Nissen 1/98	–	Mean 5.3 (3.2)	–	–	98/98	–
Greenwald, 2010	–	–	–	–	–	–	4 (3.6)	7/77	–	45/77	13/77
Sengupta, 2015	0	0	16	3	0	–	–	–	6, 1 (indefinite for dysplasia)	7	2
Ghorbani 2016	2	2	10	19	Surgery 2	–	4.5 [3.3] (1–14)	–	23/80	57/80	–
Suchniak – Mussari, 2017	–	6/33		33	–	–	Mean 3.3 [2.1] (0.8–7)	5/33	5/33	15/33	8/33
Ramay, 2017	–	–	–	11	–	3.0 (2.0–5.5) [1.0–12.0] {Median/IQR/Range}		–	0/40	40/40	
Trindade, 2017	–	–	18	2/18	–	–	Median 4	–	7/18	11/18	–
Trindade, 2018	–	–	–	EMR 27/27	–	–	6.1 (95 % CI 4.6–7.6)	–	5/27	22/27	–
Thota, 2018	1	1	7	35		–	5.2 [3.1]	–	11/81	49/81	21/81
Spiceland, 2019	–	–	46/ 46	23/46	–	–	≥ 3	–	15/46	25/46	T1a 6/46
Kaul, 2020	–	–	19/ 57	42/57	–	–	6.2	–	8/57	20/57	T1a 18/57, Invasive adenoCA 11/57
Fasullo, 2021	–	–	–	NR	–	20/62[1]	42/62[1]	–	36/62	19/62	7/62
Alshelleh, 2021	–	–	–	15/25	–	–	1–12 [3.6]	–	25		–

IMC, intramucosal cancer; EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; adenoCA, adenocarcinoma; APC, argon plasma coagulation; RFA, radiofrequency ablation;, PDT, photodynamic therapy; NR, not reported.
[1] Patients.

after LNSC, the pooled rates of CE-D and CE-IM were 83.6 % (95% CI [77.6–88.2]; I^2 60 %) and 54.7 % (95 % CI [47.6–61.6]; I^2 81 %), respectively. There was insufficient data to calculated pooled rates for CE-HGD.

Recurrence of dysplasia (RE-D) and intestinal metaplasia (RE-IM) – Across six studies, overall pooled rate of RE-D was 19.2 % (95 % CI [14–25.8]; I^2 78 %), (**Supplementary Fig. 2**), and RE-IM was 14.8 % (95 % CI [10.3–20.7]; I^2 41 %), (**Supplementary Fig. 3**).

Failure – Across 8 studies, the overall pooled rate of treatment failure was 23.6 % (95 % CI [19.4–28.3]; I^2 73 %). See **Supplementary Fig. 4**. The pooled rate of persistent dysplasia (including HGD and LGD) was 13 % (95 % CI [7.8–20.7]; I^2 64 %), the pooled rate of persistent IM was 31.1 % (95 % CI [23.1–40.5]; I^2 83 %) and the rate of progression to cancer was 6.3 % (95 % CI [3–12.6]; I^2 7 %).

▶ **Table 3** Study outcomes.

Study	Outcomes		Recurrence		Adverse events				Failure	Follow-up time months [SD] (Range)
	CE-D (CE-HGD, CE-LGD)	CE-IM	Dysplasia	IM	Pain	Stricture	Perforation	Dysphagia		
Dumot, 2009	CE-D 15/ 31	CE-IM 6/ 31	16/25 (LGD 6, HGD/IMC/ACA, esophagectomy, or death 10)	–	10/31 (mild chest pain 7/31, severe chest pain 3/31)	3/31	1/31	–	9/31 (HGD 8/26, IMC 1/5)	Median 12 m (6–24)
Shaheen, 2010	CE-D 52/60, CE-HGD 58/60	CE-IM 34/60	–	–	2/98 (severe chest pain)	3/98	0/98	-	–	Mean 10.5 m [8.3]
Greenwald 2010	CE-D 22/24 – 15/17 (HGD), 4/4 (IMC), 3/3 (Barrett's Carcinoma); CE-HGD – 23/24	CE-IM 14/24 – 9/17 (HGD), 3/4 (IMC), 2/3 (Barrett's Carcinoma)	–	–	Chest pain 57/323, Abdominal pain 14/323 (procedures)	3/77	1/77	43/323 (procedures)	–	9.3 m (3,13) – 13.8 m (10,18)
Sengupta, 2015	CE-D 12/ 16	CE-IM 5/ 16	after CE-D 0/12	–	–	3/21	1/21	3/21	–	Median 7.5 m (2–25 m)
Ghorbani 2016	CE-D 67/80 [HGD: CE-HGD 52/57, CE-D 46/57; LGD: CE-D 21/23]	CE-IM 51/80 [HGD: CE-IM 37/57; LGD: CE-IM 14/23]	–	–	28/96 (Mild-mod abdominal pain 6/96, CP 22/96)	1/96	0/96	11/96	–	Mean 21 m (12–24 m)
Suchniak – Mussari, 2017	CE-D 17/20 (CE-HGD 14/15, CE-LGD 3/5); CE-IMC + D – 6/8	CE-IM 16/33	–	–	–	5/45	0/45	–	17/33 (IM 13/33, LGD 1/33, HGD 1/33, IMC 2/33)	Mean 27.6 m [13.2]
Ramay, 2017	CE-D: 27/36 (CE-HGD 32/39)	CE-IM 17/26	after CE-D: 7/39 (HGD)	–	–	–	–	–	13/40 (LGD 3/40, IM 10/40)	Mean 69.7 m [13.8] (53.8–106.0)
Trindade, 2017	CE-D 13/18 (CE-LGD 3/4, CE-HGD 5/7)	CE-IM 9/ 18	after CE-D 0/18	after CE-IM 0/18	0/18	0/18	–	–	–	4.25 m (3–11) [Median]
Trindade, 2018	CE-D 22/27	CE-IM 19/27	–	after CE-IM 9/ 27	0/27	0	0	0	8/27 (2/27 LGD, 3/27 IM, 3/27 cancer)	24 m (12–660 [Median]
Thota, 2018	CE-D 63/81	CE-IM 33/81	–	after CE-IM 9/63	–	–	–	–	10/81 (progression/failure)	31.8 m [12.6, 50.7] [Median]
Spiceland, 2019	CE-D 38/46	CE-IM 21/46	–	–	–	3/46	–	–	8/46 (1/46 cancer, 2 lost to follow up, 5 undergoing treatment)	18m-22 m

▲ Table 3 (Continuation)

Study	Outcomes		Recurrence		Adverse events			Failure	Follow-up time months [SD] (Range)
	CE-D (CE-HGD, CE-LGD)	CE-IM	Dysplasia	IM	Pain	Stricture	Perforation	Dysphagia	
Kaul, 2020	CE-D –51/52	CE-IM – 39/52; LGD 6/7; HGD 13/17; T1a 13/17; inv EAC 7/11	4/34 after CE-IM; LGD 2/34, HGD 2/34	after CE-IM 3/34	0/57	0/57	1/57	0/57	20/39 (focal IM)	Mean 57.5 m
Fasullo, 2021	CE-D 44/62	CE-IM 41/62	6/44 after CE-D	after CE-IM 6/41	0/62	0/62	0/62	–	11/62 (persistent dysplasia/progression)	12 m
Alshelleh, 2021	CE-D 24/25	CE-IM 20/25	–	–	–	3/25	–	–	–	Mean 9–18 m [15]

LGD, low-grade dysplasia; HGD, high-grade dysplasia; IM, intestinal metaplasia; EAC, esophageal adenocarcinoma; IMC, intramucosal carcinoma; CE-D, complete eradication of dysplasia; CE-IM, complete eradication of intestinal metaplasia.

Safety outcomes

Pooled incidence of post therapy strictures – Incidence of strictures was reported in 12 studies. The overall pooled rate of post-cryotherapy strictures was 4% (95% CI [2.7 – 5.9]; I^2 2%) (**Supplementary Fig. 5**).

Pooled incidence of post therapy perforation – The incidence of perforations was reported in 9 studies. The overall pooled rate of perforation was 0.8% (95% CI [0.3–2.1]; I^2 0%) (**Supplementary Fig. 6**).

Pooled incidence of post therapy pain – the incidence of post therapy chest and/or abdominal pain was reported in seven studies. The overall pooled rate of post procedure pain was 10.3% (95% CI [7.6–13.7]; I^2 64%). Mild chest pain was reported by 29 patients. Mild-moderate abdominal pain was reported in seven patients. Severe chest pain occurred in five patients (**Supplementary Fig. 7**).

Validation of meta-analysis results

Sensitivity analysis

To assess whether any one study had a dominant effect on the meta-analysis, we excluded one study at a time and analyzed its effect on the main summary estimate. We found that exclusion of any single study did not significantly affect our primary outcomes (**Supplementary Fig. 8a to 8c**).

Heterogeneity

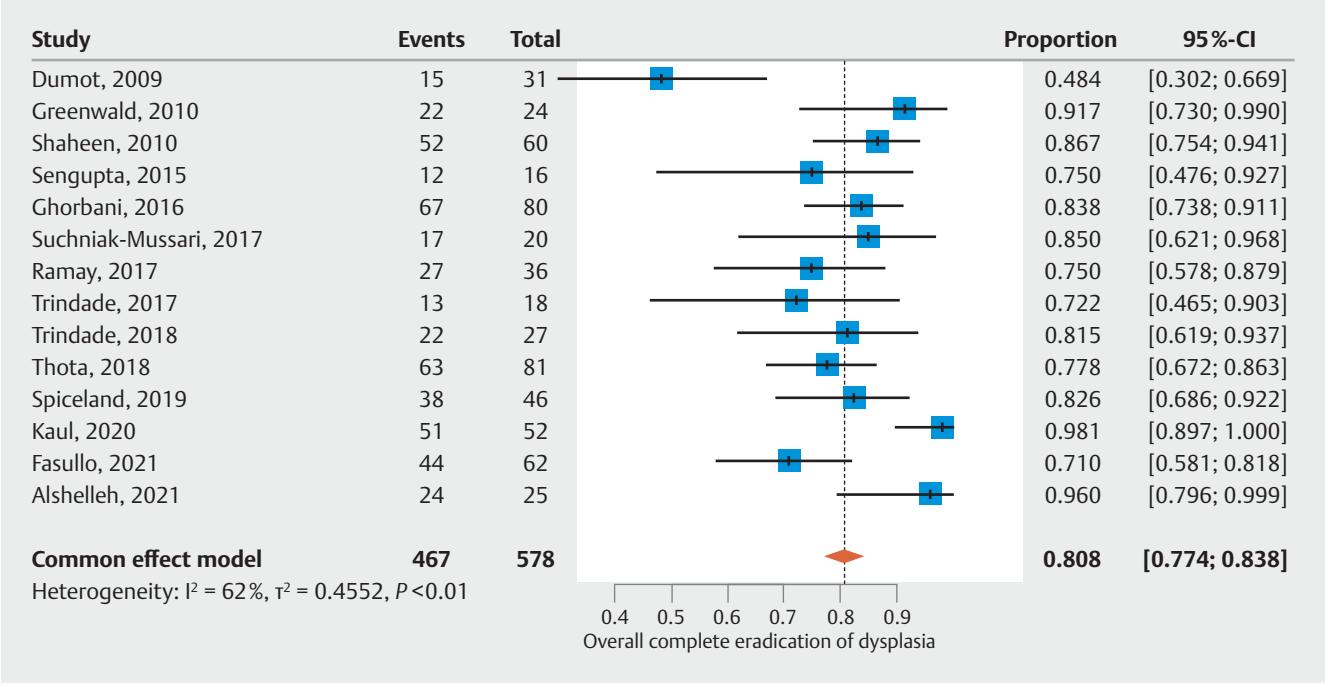
We assessed dispersion of the calculated rates using the I^2 percentage values as reported in the meta-analysis outcomes section. We found moderate to substantial heterogeneity in our overall pooled outcomes and low to substantial heterogeneity in our subgroup analysis. Further assessment of pooled analysis revealed that exclusion of the study by Dumot et al, resulted in significant decrease in heterogeneity for pooled rates of CE-D, CE-IM and RE-D. The patient population in this study primarily included patients with BE-HGD and IMC, resulting in lower rates of CE-D and CE-IM. The overall high heterogeneity can likely be explained by variation in the technique of cryotherapy, including number and duration of each freeze cycle as well as the interval time allowed for thawing, variability in BE length and a wide range of follow up period among the included studies.

Publication bias

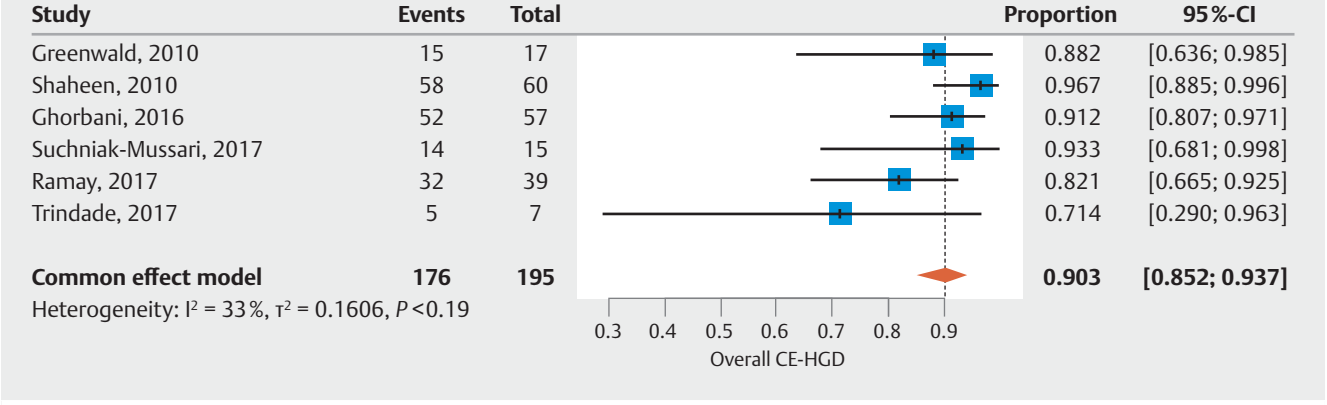
Based on visual inspection of the funnel plot for our study outcomes, we found no evidence of publication bias. Quantitative assessment demonstrated an Egger's 2-tailed *P* values of 0.05 and 0.63 for our primary outcomes, CE-D and CE-IM, respectively (**Supplementary Fig. 9a** and **b**).

Discussion

Our analysis, the largest one to date, shows that pooled rates of CE-D, CE-HGD and CE-IM with liquid nitrogen spray cryotherapy are 80.8%, 90.3% and 55.8%, respectively. In LNSC naïve patients with prior history of endoscopic resection for nodular BE, pooled rates of CE-D and CE-IM were 79.9% and 67.1%, respectively. Among patients with mean follow up over 24 months, rates were 83.6% and 54.7%. In terms of safety, pooled rates of post LNSC strictures and perforation were 4%



▶ **Fig. 1** Forest plot, overall pooled rate of CE-D.

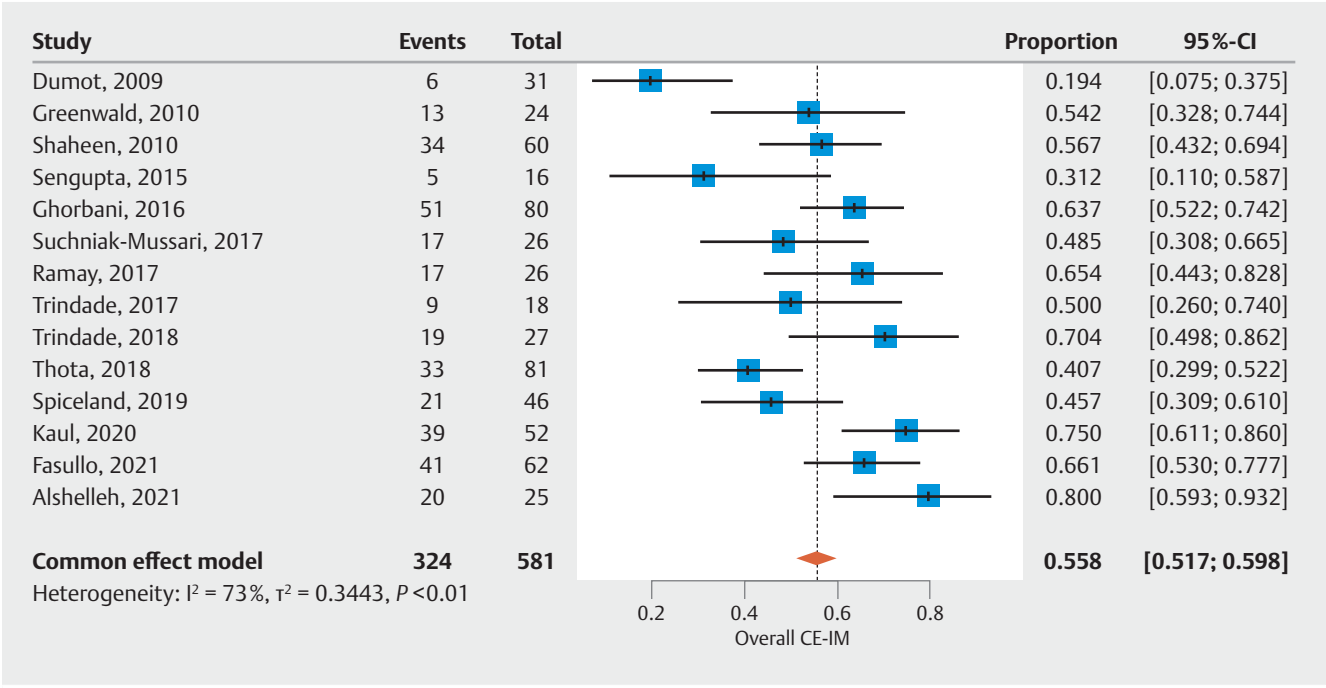


▶ **Fig. 2** Forest plot, overall pooled rate of CE-HGD.

and 0.8%, respectively, which are similar to what have been previously reported for RFA. [41] Our analysis suggests that liquid nitrogen spray cryotherapy is an acceptable treatment for BE in both ablation naïve and treatment experienced patients.

Given the presence of level I evidence documenting superiority over endoscopic surveillance and the large number of publications documenting efficacy in a variety of treatment settings, societal guidelines recommend RFA as first-line therapy for ablation of flat-type dysplastic BE or BE after resection of visible lesions [5, 42, 43]. Long-term follow-up data after RFA has shown that patients treated with ablation for dysplastic BE have >30% chance of having recurrent disease within 5 years. Studies have shown that during follow up ranging from 0.2 to 5.8 years, 32% patients have recurrence of BE or dysplasia, and 17% have a recurrence of dysplasia [44]. Some reported predictors of disease recurrence are older age, non-Caucasian race and longer length of pretreatment BE [45]. In our analysis, while we were unable to assess for predictors of recurrence following LNSC, we found that across six studies, pooled rates of dysplasia recurrence after CE-D and IM recurrence after CE-IM, were 19.2% and 14.8%, respectively. Follow up time in these studies ranged from 4.25 months [33] to 69.7 months [32].

The rate of esophageal strictures is estimated to be about 5.1% following RFA and 13.3% in cases where endoscopic resection is performed concurrently with RFA [41]. We found that among 14 studies, including those in which patients underwent EMR and/or ESD along with cryotherapy, only 24 of 603 patients (3.9%) had post therapy strictures, most of which responded to endoscopic dilation. It is unclear however, whether these strictures developed secondary to LNSC or from history



▶ **Fig. 3** Forest plot, overall pooled rate of CE-IM.

of prior ablations and/or endoscopic resection. Similarly, post procedure pain (defined as significant pain requiring medical attention or treatment) has been reported to occur in 3.8 % patients following RFA [41]. We found that the overall pooled rate of post procedure pain was 10.3 %, with severe pain only being reported by five patients. While we did not aim to compare outcomes of cryotherapy to RFA, our analysis sheds light on the safety profile and recurrence rates of CE-D and CE-IM following LNSC over a large patient cohort and long follow up time.

Incidence of EAC in BE patients following RFA is estimated to be about 1 % with mean follow up of 2.7 years [46]. A more recent study by van Munster et al on long term outcomes of RFA (with or without endoscopic resection) for Barrett's neoplasia, reported a 6 % (78/1348) failure rate, defined as patients with remaining Barrett's mucosa and/or dysplasia, after a median of 10 months (range 5–22 months) [47]. Prior studies on cryotherapy for BE have reported pooled rates of persistent IM and dysplasia as 13.7 % and 7.3 %, respectively [18]. In our analysis, we found that the overall pooled failure rate, defined as defined as lack of response to therapy, demonstrated by persistence of the previously diagnosed intestinal metaplasia, dysplasia, or cancer, or progression to worsening dysplasia or cancer, was 23.6 %. Persistent dysplasia, including HGD and LGD, was seen 13 %, IM in 31.1 % and progression to cancer occurred in 6.3 % patients. Two studies did not report failure rates separately as persistence of dysplasia/IM or progression to cancer. Thota et al reported 12.3 % (10 of 81 patients) and Fasullo et al reported 17.7 % (11 of 62 patients) as failures. Higher rates of failure in our analysis may also be because pooled rates were calculated from studies including 491 patients with either HGD and/or IMC and 11 patients with invasive adenocarcinoma. Our analysis suggests that despite acceptable pooled rates of CE-D, CE-HGD and CE-IM, patients must be closely monitored for dysplasia/IM recurrence and/or progression to EAC during follow up.

Our analysis has several strengths. First, our analysis included only those studies where cryotherapy was performed using liquid nitrogen. We conducted a systematic literature search with well-defined inclusion criteria, careful exclusion of redundant studies with potential patient overlap, inclusion of good quality studies with detailed extraction of data and rigorous evaluation of study quality. We did not include any conference abstracts in our analysis. Second, in addition to reported overall pooled results, we performed sub-group analysis based on patients' treatment history and assessed durability of response with variation in mean/median follow up times. This was done since eradication rates are expected to differ in patients undergoing cryotherapy as primary vs salvage therapy. Our study also has several limitations, most of which are inherent to any meta-analysis. First, we were unable to definitively rule out the possibility of patient overlap, especially among multi-center studies. We were unable to assess our outcomes based on length of Barrett's segment and number of LNSC sessions per patient. There was variability in the number and duration of LNSC cycles performed among studies, which likely explains the heterogeneity in some of our outcomes. In one of the studies included in our analysis, 6 patients underwent balloon based cryotherapy [48]. Kaul et al included 11 of 57 (19.3 %) patients with invasive adenocarcinoma, where given the risk of metastasis, combination endoscopic resection and ablation therapy is recommended. [49] Of these, seven patients (63.6 %) achieved CE-IM. Additionally, the high rate of failures (20 of 39) was due to persistent fo-

cal IM. Several studies included in our analysis were retrospective in design which may have contributed to selection bias.

Nevertheless, our analysis is the largest till date in assessing the safety and efficacy of liquid nitrogen-based spray cryotherapy in patients with Barrett's neoplasia. We found that pooled rates of dysplasia and IM eradication in patients with follow up longer than 24 months are acceptable. The rates of post therapy strictures and perforation are similar to those reported with RFA. Despite these results, rates of persist dysplasia, IM and progression to cancer must be taken into consideration while selecting patients for LNSC. Further studies with longer follow-ups are needed to validate our findings.

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Competing interests

The authors declare that they have no conflict of interest.

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