Care Seeking for Neonatal Illness in Low- and Middle-Income Countries: A Systematic Review

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Abstract

Background: Despite recent achievements to reduce child mortality, neonatal deaths continue to remain high, accounting for 41% of all deaths in children under five years of age worldwide, of which over 90% occur in low- and middle-income countries (LMICs). Infections are a leading cause of death and limitations in care seeking for ill neonates contribute to high mortality rates. As estimates for care-seeking behaviors in LMICs have not been studied, this review describes care seeking for neonatal illnesses in LMICs, with particular attention to type of care sought.

Methods and Findings: We conducted a systematic literature review of studies that reported the proportion of caregivers that sought care for ill or suspected ill neonates in LMICs. The initial search yielded 784 studies, of which 22 studies described relevant data from community household surveys, facility-based surveys, and intervention trials. The majority of studies were from South Asia ($n = 17/22$), set in rural areas ($n = 17/22$), and published within the last 4 years ($n = 18/22$). Of the 9,098 neonates who were ill or suspected to be ill, 4,320 caregivers sought some type of care, including care from a health facility ($n = 370$) or provider ($n = 1,813$). Care seeking ranged between 10% and 100% among caregivers with a median of 59%. Care seeking from a health care provider yielded a similar range and median, while care seeking at a health care facility ranged between 1% and 100%, with a median of 20%. Care-seeking estimates were limited by the few studies conducted in urban settings and regions other than South Asia. There was a lack of consistency regarding illness, care-seeking, and care provider definitions.

Conclusions: There is a paucity of data regarding newborn care-seeking behaviors; in South Asia, care seeking is low for newborn illness, especially in terms of care sought from health care facilities and medically trained providers. There is a need for representative data to describe care-seeking patterns in different geographic regions and better understand mechanisms to enhance care seeking during this vulnerable time period.

Please see later in the article for the Editors' Summary.

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Abbreviations: LMIC, low- and middle-income country.

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Introduction

As the field of public health continues to strive to reach the fourth United Nations Millennium Development Goal (MDG) to reduce child mortality, a systematic analysis of the progress made indicates that there has been limited advancement to reduce the rate of neonatal mortality [1]. While the mortality of children under five years of age has decreased worldwide from 10.8 million deaths per year in 2000 to 8.8 million deaths in 2008, neonatal deaths decreased only from 3.9 million to 3.6 million during that time. This number represents an increased proportion of neonatal to under-five child deaths from 36% in 2000 to 41% in 2008 [1]. Of these deaths, over 90% occur in low- and middle-income countries (LMICs), making the risk of death in the neonatal period in LMICs more than six times the risk in high-income countries [2].

Of the several contributors to neonatal mortality, infections are the single largest cause of death, responsible for an estimated 25% of all neonatal deaths [3]. Neonatal sepsis has traditionally been defined as bacteremia with hemodynamic compromise and systemic signs of infection. However, in LMICs, continuous vital sign monitoring, blood cultures, and other confirmatory laboratory tests may not be available and thus the diagnosis of neonatal sepsis is often based on clinical signs. These signs are frequently nonspecific and may include lethargy or irritability, poor feeding, vomiting, respiratory distress, apnea, fever, and hypothermia [4]. Inappropriate and delayed care seeking can contribute substantially to the resulting neonatal mortality.

Effective strategies to improve survival from neonatal infections in LMICs require a clear understanding of neonatal care-seeking behaviors and patterns by caregivers. Care-seeking behaviors are often described in the literature by either the type of health care facility or provider to which the neonate presented. Additionally, to deliver successful neonatal health care interventions, health care facilities and providers must not only be available and accessible, but illness must first be recognized and care desired by the neonate's caregiver, often a parent or other family member. As such, understanding care-seeking practices becomes essential for health interventions to have a positive impact.

The overall aim of this literature review is to describe the proportion of caregivers who seek medical care once they recognize their neonate is ill or is suspected to be ill in LMICs. This review provides data to model the potential impact of point of care testing for neonatal infections, given the state of current care-seeking patterns in LMICs.

Methods

A systematic literature search was conducted between September and October, 2011 of the following databases: PubMed/Medline [5], Embase [6], the Cochrane Library [7], the Global Health Library [8], African Index Medicus [9], African Trials Register [10], Africa-Wide Information [11], and Literature on the Health Sciences in Latin America and the Caribbean (LILACS) [12]. "Grey" literature sources were searched to identify program and unpublished reports, including search engines Eldis [13], Data Online for Population, Health and Nutrition (DOLPHN) [14], Reproductive Health Gateway [15], donor websites (Basic Support for Institutionalizing Child Survival [BASICS] [16] and Saving Newborn Lives [17]), Demographic and Health Surveys (DHS) [18], and Service Provision Assessments (SPA) [19]. Search terms were generated using key words and mesh headings for care seeking, neonates, and LMICs. See Texts S1 and S2 for the study's PRISMA checklist and protocol. A complete list of search terms, formatted for PubMed, is provided in the study protocol. The review is registered with PROSPERO (registration number CRD42011001654) [20].

Database searches were not limited by date. Language restrictions were limited to those articles published in the Latin alphabet and articles that required translation to English were translated by the authors. Additional articles were identified from the bibliographies of the articles reviewed through snowball sampling [21]. All citations were imported into an electronic database (Refworks, Proquest). Two reviewers independently assessed the studies identified during the screening search using inclusion/exclusion criteria.

Inclusion/Exclusion Criteria

Studies were included if they met the following inclusion criteria: (1) the publication was a study of original work; (2) the study was conducted in a LMIC, using the World Health Organization's (WHO) definition of LMICs [22]; (3) the study quantitatively specified care seeking for neonates, defined as an infant less than or equal to 28 d of age. If the study did not specify the exclusion of neonates from the study, corresponding authors were contacted for further clarification of their study sample; (4) the study specified that care was sought for an illness or a suspected illness, opposed to non-illness-related complications, such as prematurity, intrapartum complications, tetanus, or congenital abnormalities.

Articles were excluded if they did not specifically identify neonates, specify care-seeking behaviors, or occur in a LMIC. In terms of study design, four types of publications and studies were excluded: (1) review articles or editorials, because they did not provide primary data; (2) intervention studies that offered care in the home setting and explored themes of care acceptance, because acceptance of care in a trial scenario was considered to be beyond the scope of this care-seeking review; (3) qualitative studies that described determinates of care seeking and not care-seeking events, which was also considered beyond the scope of this review; and (4) publications that described a duplicate study population. If this occurred, the subsidiary publication was excluded.

Definitions

Neonatal illness was defined according to the definitions provided by the included studies. Care seeking was defined as any care sought for a neonate that was perceived by the caregiver to be ill, sick, or septic. A caregiver was defined as the individual who sought care for the ill neonate, as identified by the study, and was often a family member, such as a mother or grandmother. Type of care sought was categorized according to facility type, health care provider type, home care, or no care. Facility type was categorized as either primary or secondary health centers or pharmacies, as identified by each study. Primary health centers included public and private clinics, health centers, and out-patient care; secondary health centers included public and private hospitals and health facilities with in-patient care. Health care providers included medically trained providers and unqualified providers. Medically trained providers included government providers (GPs), who were qualified medical practitioners employed at government hospitals, nongovernmental consultants (NGCs), who were health care providers that worked through privately owned clinics and hospitals, and paramedics [23]. Unqualified providers included unqualified village doctors, traditional healers, spiritual healers, unqualified allopaths, homeopaths, and nongovernmental dispensers (NGDs), defined as selfemployed health care providers who could dispense medicines without prescriptions [23]. Home care included any care provided by family members or neighbors in a home setting.

Data Extraction and Assessment of Study Quality

From each included study the following were abstracted: study location, design, setting, the number of subjects included in the study population, the number of neonates with an illness or suspected illness, the number of caregivers that sought care, and where care was sought. To facilitate a comparison of where care was sought, type of care was grouped in terms of facility or provider type. All care-seeking encounters were extracted from the data and presented in this review, including multiple types of care sought by one caregiver. If the study did not specify the number of neonates with an illness or a suspected illness or did not provide the number of caregivers that sought care, corresponding authors were contacted to obtain this information directly.

Study quality was assessed on the basis of a modification of methods for systematic reviews for intervention effectiveness described by the Child Health Epidemiology Reference Group (CHERG), using principles relevant for the aims of this particular review [24]. Each study was evaluated by study design, population representativeness, the quality and consistency of definitions, generalizability to the population of interest, and precision in terms of the number of reported care-seeking events. "Study design" was defined according to whether data were prospectively or retrospectively collected and potentially influenced by recall bias. "Population representativeness" described the extent to which the study sample was representative of the general population as being either population or health facility based with minimal or moderate bias. "Quality of definitions" described the extent to which a study defined neonate illness, care seeking, type of health care sought. "Consistency" was assessed across all studies to ascertain the extent to which these definitions were similar. "Generalizability" was defined according to the degree to which results could be applied to other settings and populations of interest. "Precision" was defined as the extent to which the study populations included a sufficient sample size. If a study's total study population was less than 50, the quality of the evidence was considered insufficient to generalize the effect of the outcome to the target population [24].

Data Synthesis

Due to study heterogeneity, a meta-analysis was not possible. Therefore, we reported the literature estimates and described those data narratively. To minimize the inherent selection bias of facility-based surveys regarding their population sample and care-seeking behaviors, we did not include studies that were facility-based in our description of the total number of ill neonates, the total number of care-seeking events, and the type of care sought. Additionally, when describing data from intervention trials, including randomized control trials (RCT) and before/after interventions, we included data pertaining to neonates who were either in the control or "before" groups to minimize the interventions' effect on caregivers' initial care-seeking behaviors.

Results

Our initial search yielded 784 citations; after excluding duplicate articles and review of studies' titles and abstracts, 211 articles were selected for full-text review (Figure 1). Of these, 155 articles were excluded on the basis of exclusion criteria. Two additional studies were identified by bibliography review. Of the remaining 58 articles, 41 articles did not specify exclusion of neonates or required additional information pertaining to the number of ill neonates and/or care-seeking events; of these, the corresponding authors of 39 studies were contacted for further information (two authors could not be located). Twenty-four authors responded to our inquiry, providing the necessary information to apply our inclusion and exclusion criteria.

A total of 22 articles met our inclusion criteria, of which 11 studies were community-based surveys, seven were intervention trials, and four were facility-based surveys (Table 1). The majority of the studies occurred in the Southeast Asia Region ($n = 17/22$). The remaining five studies occurred in Africa. All studies were published after 2000 and the majority were published after 2007 ($n = 18/22$). Thirteen studies were cross-sectional surveys, of which 11 were community based and two were facility based. Five studies were cluster RCTs (c-RCT), three were "before and after" interventions, and one was a prospective follow-up study. The majority of studies were conducted in rural settings ($n = 17/22$); of the remaining studies, three occurred in urban settings, one in a peri-urban setting, and one in both urban and rural settings.

Percentage of Sick Newborns Seeking Care Outside of the Home

The included studies identified a total of 9,680 neonates who were ill or suspected of being ill. After controlling for facility-based studies, 9,098 ill neonates were identified in community-based studies as being ill or suspected of being ill, of which there were a total of 4,320 care-seeking events. Among the non-facility-based studies, 370 care-seeking events occurred at a health care facility and 1,813 occurred with a provider. Care seeking by caregivers for any care ranged between 10% and 100%, with a median of 59%. Care seeking from a health care provider yielded the same range and median, while care seeking at a healthcare facility ranged between 1% and 100%, with a median of 20%.

All community-based intervention studies occurred in the Southeast Asia Region and used interventions such as women's groups and action learning cycle ($n = 3/7$) [25–27], essential newborn care (ENC; $n = 2/7$) [28,29], behavior change and illness recognition ($n = 1/7$), birth preparedness (BPP; $n = 1/7$) [30]. All studies showed an increase in care seeking following the given intervention.

Quality Assessment

While all studies described caregivers that sought some form of care, eight studies described caregivers who sought care from a facility and 12 describe care sought from a provider. Table 2 presents a summary of data for these care-seeking categories, while Table 3 describes the quality of each individual study. Of the 22 studies, more than half of the studies were retrospective ($n = 14$), had recall periods of less than 2 mo ($n = 13$), and were population based with minimal bias ($n = 14$). Four studies did not define neonatal illness and two studies did not define care seeking or the type of care sought. In general, the studies reported a wide range of definitions to define neonatal illness. Illness definitions ranged from illness diagnosed by documenting one or more clinical danger signs or complication, to specific danger signs (such as sepsis or pneumonia; the presence of a cough, fever, or diarrhea; or the presence of jaundice), to not being defined. Definitions of care seeking ranged from any care, to care sought outside the home or from a specific health care facility or provider, to not being defined. While two studies did not state their sample size, only one study had a sample size of less than 50 neonates. Because of the inherent selection bias of facility-based surveys regarding their population sample and their care-seeking behaviors, facility-

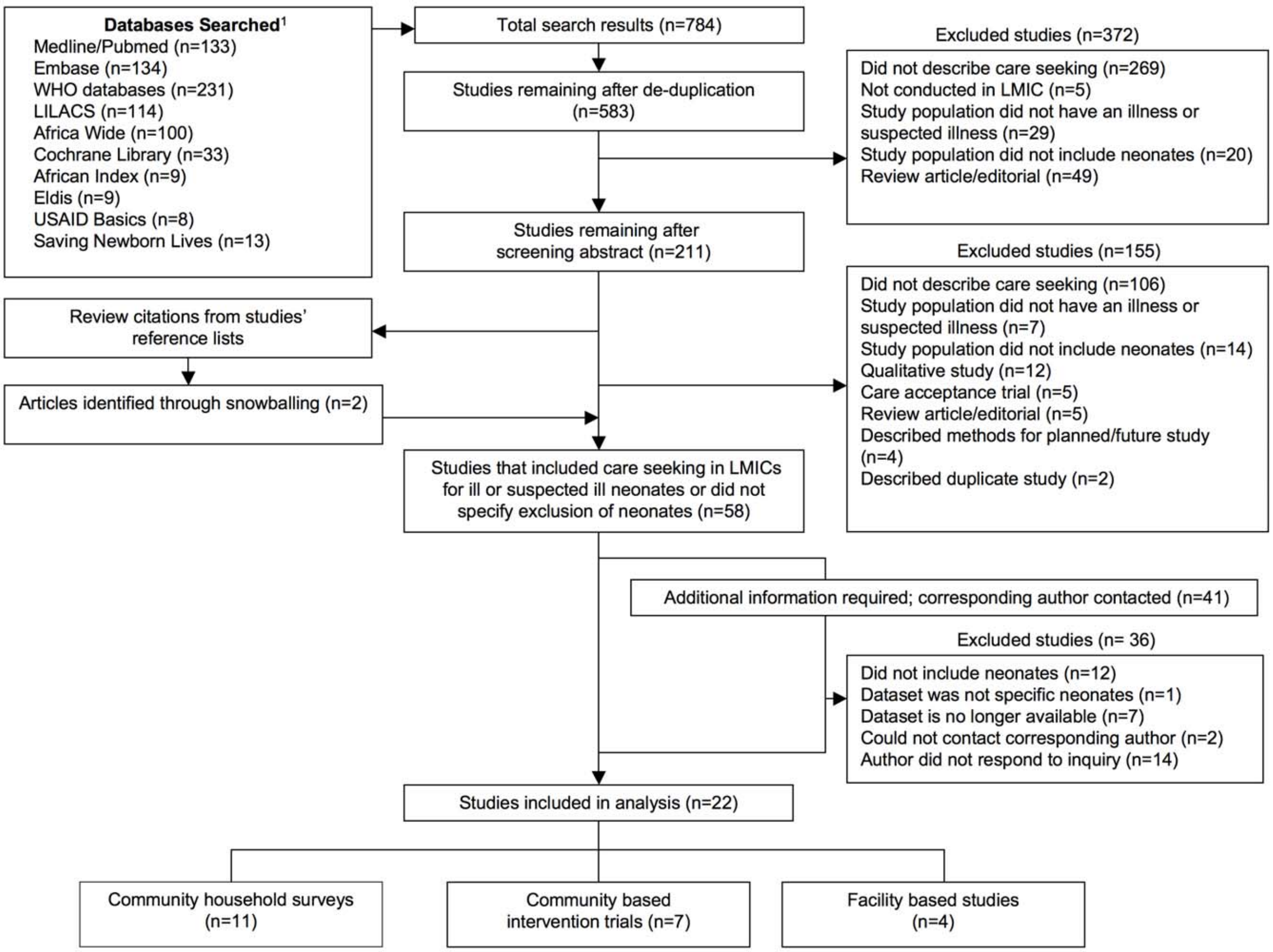

Figure 1. Literature flowchart of care seeking for ill or suspected ill neonates. Footnote 1: The following databases did not yield any search results: UNICEF, Reproductive Health Gateway, Dolphn, DHS, SPA, and African Trials.
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based studies were excluded from further descriptions regarding type of care sought.

Type of Medical Attention/Care Sought for Sick Newborns

Table 4 presents findings from studies that reported a specific type of medical care sought and further delineating care in terms of type of facility and provider, homecare, and no care. Three studies did not identify specific type of care and were not included in this table [27,30,31]. Six studies reported that care sought at a secondary health center ranged from 4% to 66%. Two studies reported care sought at a primary health center (12% and 83%, respectively). Two studies reported 1% and 33% of care was sought at a pharmacy. In terms of type of health provider, nine studies reported care sought from a medically trained provider ranged from 4% to 100%. Seven studies reported care sought from an unqualified provider ranged from 1% to 83%.

Discussion

While neonatal deaths comprise a staggering and increasing proportion of global deaths among children under the age of five, our review identified a paucity of data regarding care-seeking behaviors for newborn illnesses in LMICs. In LMIC settings, approximately three-quarters of neonatal deaths occur in the first week of life and nearly half occur in the first 24 h, of which more than half occur at home [32]. Timing is critical to providing neonates with appropriate care at the onset of illness and delays in the decision to seek care can have significant consequences [33]. Thus prioritizing timely and adequate care seeking for illnesses in LMICs is an essential component to improving neonatal health.

As illustrated in Figure 2, the caregiver faces multiple decision points once the neonate displays signs of suspected illness. First, they must be able to recognize these signs; while illness recognition is fundamental in the decision to seek care, this can be particularly challenging in the neonate due to the lack of specific symptoms [4,34,35]. Other studies have described interventions to promote maternal recognition of neonatal illnesses [34,36,37]. Our review builds on this work to describe care seeking as the next step in this process of treating neonatal illnesses to reduce neonatal mortality. Understanding baseline care seeking becomes of particular importance if we were to enhance illness recognition at the home through the development of a possible point of care diagnostic test, which would then inform the caregiver as to when a neonate is ill enough to require care to be sought.

Our review found the majority of the studies occurred in rural areas in South Asia suggesting findings may not be generalizable to urban settings or other geographic regions and highlighting

Table 1. Characteristics of studies that describe care seeking for ill or suspected ill neonates.

Primary Author, Year	Country	Rural/Urban	Study Design	Study Setting	Study Sample (*n*)	Neonates with Illness/ Suspected Illness (n)	*n* Caregivers Who Sought Care by Type of Care Sought (%)[a]
Community Household Surveys							
South Asia							
Ahmed, 2001 [55]	Bangladesh	Rural	Cross-sectional community survey	4 rural subdistricts, Chittagong and Jessore	1511,511	740	Any care: 644 (87%); From facility: 30 (4%); From provider: 614 (82%)
Bhandari, 2002 [56]	India	Urban	Cross-sectional community survey	Urban slum, Delhi	21	9	Any care: 2 (22%); From facility: 2 (22%)
Mohan, 2008 [57]	India	Rural	Cross-sectional community survey	Rural district, Rajasthan	290	202	Any care: 63 (31%); From facility: 24 (12%); From provider: 20 (10%)
Baqui, 2008 [58][b]	Bangladesh	Rural	Cross-section community survey, nested in C-RCT	Sylhet district	Baseline: 5,577	Baseline: 1,226	Baseline: Any care: 498 (41%); From provider: 498 (41%)
Dongre, 2008 [59]	India	Rural	Cross-sectional community survey	Rural district, Wardha	1160	321	Any care: 134 (42%); From facility: 56 (17%)
Dongre, 2009 [60]	India	Urban	Cross-sectional community survey	Field practice area, Wardha	72	27	Any care: 27 (100%); From provider: 27 (100%)
Willis, 2009 [61]	India	Rural	Cross-sectional community survey	Rural block, Uttar Pradesh	255	158	Any care: 120 (76%); From provider: 62 (39%)
Chowdhury, 2011 [62]	Bangladesh	Rural	Cross-sectional community survey	Rural subdistrict, Matlab	365	365	Any care: 228 (62%); From facility: 2 (1%); From provider: 226 (62%)
Africa							
Bazzano, 2008 [63]	Ghana	Rural	Cross-sectional community survey	3 villages and 1 town, Kintampo District	2,878	59	Any care: 36 (61%); From provider: 36 (61%)
Manji, 2009 [31]	Tanzania	Urban/rural	Cross-sectional community survey	N/A	N/A	N/A	Any care: N/A (100%)
Waiswa, 2010 [64][2]	Uganda	Rural	Cross-sectional health center survey	16 health facilities	64	6	Any care: 6 (100%); From facility: 6 (100%)
Community Based Intervention Studies							
South Asia							
Manandhar, 2004 [26]	Nepal	Rural	C-RCT	42 clusters, Makwanpur district	Control: 3,226	Control: 1,320	Control: Any care: 131 (10%); From facility: 131 (10%);
					Intervention: 2,899	Intervention: 919	Intervention: Any care: 219 (24%); From facility: 219 (24%)
Kumar, 2008 [28]	India	Rural	C-RCT	Household survey	Control: 988	Control: 296	Control: Any care: 254 (86%); From provider: 235 (80%)
					ENC: 1,458	ENC: 319	ENC: Any care: 257 (81%); From provider: 230 (73%)
					ENCT: 1,039	ENCT: 227	ENCT: Any care: 204 (89%); From provider: 182 (80%)
McPherson, 2008 [30]	Nepal	Rural	Before/after intervention	106 rural villages, Siraha District	N/A	N/A	Before: Any care: N/A (78%); After: Any care: N/A (85%)

Table 1. Cont.

Primary Author, Year	Country	Rural/Urban	Study Design	Study Setting	Study Sample (*n*)	Neonates with Illness/ Suspected Illness (n)	*n* Caregivers Who Sought Care by Type of Care Sought (%)[a]
Dongre, 2009 [65]	India	Rural	Before/after intervention	23 villages, Wardha	Before: 404	Before: 246	Before: Any care: 119 (48%); From facility: 119 (48%)
					After: 393	After: 147	After: Any care: 114 (78%); From facility: 114 (78%)
Azad, 2010 [27]	Bangladesh	Rural	C-RCT	Three rural districts, Bogra, Faridpur, Moulavibazar	Control: 3,227	Control: 923	Control: Any care: 244 (24%)
					Intervention: 3,162	Intervention: 866	Intervention: Any care: 195 (23%)
Darmstadt, 2010 [29]	Bangladesh	Rural	C-RCT	Rural subdistrict, Mirzapur	Control: 5,241	Control baseline: 812	Control baseline: Any care: 763 (94%); From provider: 222 (27%)
						Control endpoint: 400	Control endpoint: Any care: 384 (96%); From provider: 138 (35%)
					Intervention: 4,616	Intervention baseline: 733	Intervention baseline: Any care: 682 (93%); From provider: 225 (31%)
						Intervention endpoint: 355	Intervention endpoint: Any care: 344 (97%); From provider: 198 (56%)
Tripathy, 2010 [25]	India	Rural	C-RCT	36 clusters, Jharkhand and Orissa	Control: 8,867	Control: 2,388;	Control: Any care: 1050 (44%)
					Intervention: 9,468	Intervention: 1,739	Intervention: Any care: 940 (54%)
Facility-Based Studies							
South Asia							
Awasthi, 2006 [66]	India	Rural	Cross-sectional hospital survey	2 public hospitals, Lucknow	200	79	Any care: 46 (56%); From provider: 46 (56%)
Awasthi, 2008 [67]	India	Urban	Prospective follow-up study	Urban city, Lucknow	289	79	Any care: 64 (81%); From provider: 64 (81%)
Awasthi, 2009 [23]	India	Urban	Before/after intervention	Rural village, Sarojininagor Block	510	Before: 242	Before: Any care: 196 (81%); From provider: 111 (46%)
						After 217	After: Any care: 192 (89%)
Africa Region							
Ogunlesi, 2011 [68]	Nigeria	Urban	Cross-sectional health center survey	1 public hospital, Sagamu	182	182	Any care: 144 (79%); From facility: 144 (79%)

[a]Percentage reported as a proportion of all neonates with an illness or suspected illness reported by each study.
[b]Data obtained via correspondence with corresponding author.
Abbreviations: C-RCT, cluster randomized controlled trial; CHW, community health worker; ENC, essential newborn care; ENCT, essential newborn care plus thermostat; N/A, not available.
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Table 2. Quality of data regarding care seeking for ill or suspected ill neonates: summary of included studies.

n Studies	Study Design		Population Representativeness	Definitions of Illness, Care Seeking, and Type of Health Care		Generalizability to Population of Interest	Precision: Study Sample with >50 Neonates
	Prospective Versus Retrospective	Recall Period		Quality of Definitions	Consistency		
Studies that describe caregivers that sought any care							
22 [23,25–31,55–68]	Prospective ($n=9$); Retrospective ($n=14$)	≤2 mo ($n=13$); ≤1 y ($n=6$); >1 y ($n=1$); N/A ($n=2$)	Population based with minimal bias ($n=14$); Population based with moderate bias ($n=3$); Facility-based with minimal bias ($n=3$); Facility-based with moderate bias ($n=1$); N/A ($n=1$)	Defined illness ($n=18$); Defined care seeking ($n=20$); Defined type of health care ($n=20$)	Wide range of inconsistency of definitions	Rural ($n=17$); Urban ($n=3$); Peri-urban ($n=1$); Urban/Rural ($n=1$)	>50 neonates ($n=19$); ≤50 neonates ($n=1$); N/A ($n=2$)
Studies that describe caregivers that sought care at a health care facility							
8 [26,55,57,59,62,64,65,68]	Prospective ($n=3$) Retrospective ($n=5$)	≤2 mo ($n=5$); ≤1 y ($n=2$); N/A ($n=1$)	Population based with minimal bias ($n=5$); Population based with moderate bias ($n=2$); Facility-based with moderate bias ($n=1$)	Defined illness ($n=7$); Defined care seeking ($n=8$); Defined type of health care ($n=8$)	Wide range of inconsistency of definitions	Rural ($n=7$); Urban ($n=1$)	>50 neonates ($n=8$)
Studies that describe caregivers sought care from a health care provider							
12 [23,28,29,55,57,58,60–63,66,67]	Prospective ($n=3$); Retrospective ($n=9$)	≤2 mo ($n=9$); ≤1 y ($n=2$); >1 y ($n=1$)	Population based with minimal bias ($n=8$); Population based with moderate bias ($n=1$); Facility-based with minimal bias ($n=3$)	Defined illness ($n=11$); Defined care seeking ($n=12$); Defined type of health care ($n=12$)	Wide range of inconsistency of definitions	Rural ($n=9$); Urban ($n=2$); Peri-urban ($n=1$)	>50 neonates ($n=12$)

Abbreviations: N/A, not available.
doi:10.1371/journal.pmed.1001183.t002

Table 3. Quality of data regarding care seeking for ill or suspected ill neonates: all included studies.

Primary Author, Year	Study Design		Population Representativeness: Description of the Study Population	Quality of Definitions: Study Definitions of Illness, Care Seeking, and Type of Health Care	Generalizability: Rural or Urban	Precision: Study Sample Size >50 Neonates?
	Prospective or Retrospective	Recall Period				
Community household surveys						
South Asia						
Ahmed, 2001 [55]	Retrospective	2-mo recall period	**Population based with minimal bias**: Mothers identified from a sample registration system	**Illness:** a morbid condition, including difficulty breathing, fever, convulsion, diarrhea, boils, jaundice, umbilical redness/discharge, loss of weight, oral ulcer, cold, rash, or other; **Care seeking:** sought care from specific health care provider or facility; **Type of health care:** homeopath, village doctor, TH, government facility, private doctor, other	Rural	Yes
Bhandari, 2002 [56]	Retrospective	4-mo recall period	**Population based with moderate bias:** Neonatal deaths	**Illness:** pneumonia, sepsis, or meningitis; **Care seeking:** care from public hospital; **Type of health care:** public hospital	Rural	No
Mohan, 2008 [57]	Retrospective	45-d recall period	**Population based with minimal bias:** Live births in study population	**Illness:** at least one danger sign of fever, refusal to breast-feed, convulsions, lethargy, blood in stool, rapid breathing, or chest in-drawing; **Care seeking:** any care outside the home; **Type of health care:** any health care facility	Rural	Yes
Baqui, 2008 [58]	Retrospective	1-y recall period	**Population based with minimal bias:** Women had given birth within one y preceding survey	**Illness:** poor feeding, diarrhea, umbilical redness/discharge, red or discharging eyes, difficulty or fast breathing, chest in-drawing, jaundice, fever, convulsions, lack of crying, or unconsciousness; **Care seeking:** care from trained providers; **Type of health care:** trained providers (physicians, nurses, or family welfare assistants)	Rural	Yes
Dongre, 2008 [59]	Retrospective	1-y recall period	**Population based with minimal bias:** Mothers with a child <1 y of age	**Illness:** one or more IMNCI danger sign; **Care seeking:** any care; **Type of health care:** home care versus government hospital or private hospital	Rural	Yes
Dongre, 2009 [60]	Retrospective	1-y recall period	**Population based with minimal bias:** Women identified through a mapping exercise	**Illness:** not defined; **Care seeking:** care from medical doctor or TH; **Type of health care:** medical doctor or TH	Peri-urban	Yes
Willis, 2009 [61]	Retrospective	4-wk recall period	**Population based with minimal biased:** Pregnant women in study area, excluding those planning to deliver at a health care facility	**Illness:** perceived neonatal morbidities; **Care seeking:** any care; **Type of health care:** any health care resources	Rural	Yes
Chowdhury, 2011 [62]	Retrospective	2- to 6-wk recall period	**Population-based with moderate bias:** Neonatal deaths	**Illness:** fatal illness; **Care seeking:** care from health care provider; **Type of health care:** qualified provider (MBBS or paramedic), *Kabiraj*, unqualified allopath, homeopath, spiritual leader, pharmacy	Rural	Yes
Africa						
Bazzano, 2008 [63]	Retrospective	4-wk recall period	**Population based with minimal bias:** Women enrolled in Obaapa Vit A trial	**Illness:** not defined; **Care seeking:** any care outside the home; **Type of health care:** doctor/hospital versus TH	Rural	Yes
Manji, 2009 [31]	Retrospective	N/A	**N/A:** Sample population not stated	**Illness:** not defined; **Care seeking:** not defined; **Type of health care:** not defined	Urban/rural	N/A

Table 3. Cont.

Primary Author, Year	Study Design		Population Representativeness: Description of the Study Population	Quality of Definitions: Study Definitions of Illness, Care Seeking, and Type of Health Care	Generalizability: Rural or Urban	Precision: Study Sample Size >50 Neonates?
	Prospective or Retrospective	**Recall Period**				
Waiswa, 2010 [64]	Retrospective	4- to 6-wk recall period	**Population-based with moderate bias:** Neonatal deaths	**Illness:** sepsis or pneumonia; **Care seeking:** any care outside the home; **Type of health care:** health facility, drug shop, spiritual leader, friend/relative	Rural	Yes
Community based intervention studies						
South Asia						
Manandhar, 2004 [26]	Prospective	N/A	**Population based with minimal bias:** Married women 15–49 y with potential to become pregnant	**Illness:** any of the three illnesses (cough, fever, diarrhea); **Care seeking:** health facility; **Type of health care:** health facility	Rural	Yes
Kumar, 2008 [28]	Prospective	3-y recall period	**Population based with minimal bias:** Live births in study population	**Illness:** danger sign recognition; **Care seeking:** any care; **Type of health care:** nurse, doctor, unqualified medical practitioner, TH, other (family members)	Rural	Yes
McPherson, 2006 [30]	Prospective	1-y recall period	**Population based with minimal bias:** Mothers of live infants less one y of age at the time	**Illness:** not defined; **Care seeking:** not defined; **Type of health care:** not defined	Rural	N/A
Dongre, 2009 [65]	Prospective	1-y recall period	**Population based with minimal bias:** Mothers with a child less than one y	**Illness:** one or more danger sign; **Care seeking:** care from government hospital or from private hospital; **Type of health care:** government hospital or private hospital	Rural	Yes
Azad, 2010 [27]	Prospective	6 wk after delivery	**Population based with minimal bias:** Women aged 15–49 y, residing in study area, who gave birth during the study period	**Illness:** any cough, fever, diarrhea**; Care seeking:** any care; **Type of health care:** not defined	Rural	Yes
Darmstadt 2010 [29]	Prospective	CHWs assessed neonates on days 2, 5, and 8	**Population based with minimal bias:** Live births in study population	**Illness:** 16 neonatal danger signs; **Care seeking:** any care; **Type of health care:** any treatment versus doctor, nurse, welfare visitor, medical assistant, satellite clinic, family welfare center, health complex, doctor's chamber, clinic, or hospital	Rural	Yes
Tripathy, 2010 [25]	Prospective	6 wk after delivery	**Population based with minimal bias:** Live births in study population	**Illness:** cough, fever, and/or diarrhea; **Care seeking:** care from qualified provider; **Type of health care:** qualified provider	Rural	Yes
Facility-based studies						
South Asia						
Awasthi, 2006 [66]	Retrospective	Caregivers and CHWs who cared for a seriously ill neonate in past year and/or experienced a neonatal death or near death in past 2 y	**Facility-based with minimal bias:** Caregivers and CHWs who had given primary care to a newborn within six mo preceding survey	**Illness:** one or more IMNCI danger sign; **Care seeking:** any care; **Type of health care:** local medical doctor (registered or nonregistered) and traditional healer	Rural	Yes

Table 3. Cont.

Primary Author, Year	Study Design: Prospective or Retrospective	Recall Period	Population Representativeness: Description of the Study Population	Quality of Definitions: Study Definitions of Illness, Care Seeking, and Type of Health Care	Generalizability: Rural or Urban	Precision: Study Sample Size >50 Neonates?
Awasthi, 2008 [67]	Retrospective	6 wk±15 d after recruitment	**Facility-based with minimal bias:** Neonates delivered at a health center	**Illness:** one or more IMNCI danger sign; **Care seeking:** any care, including home remedies; **Type of health care:** home remedies, chemist, TH, qualified physician	Urban	Yes
Awasthi, 2009 [23]	Prospective	6–8 wk after recruitment	**Facility-based with minimal bias:** Neonates delivered at a health center	**Illness:** health related problem reported by caregiver; **Care seeking:** care from GP or NGC; **Type of health care:** GP, NGC, or NGD	Urban	Yes
Africa region						
Ogunlesi, 2011 [68]	Prospective	No recall period; neonates assessed at time of admission	**Facility-based with moderate bias:** Neonates with jaundice	**Illness:** jaundice; **Care seeking:** any care; **Type of health care:** private clinic, primary health center, general hospital, home care	Urban	Yes

Abbreviations: CHW, community health worker; GP, government provider; IMNCI, integrated management of childhood and neonatal illnesses, NGC, nongovernmental consultants; NGD, nongovernmental dispensers; N/A, not available; PHC, primary health care; SHC, secondary health care; TH, traditional healer.

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substantial gaps in the literature. Of the available data, the results vary greatly: studies showed anywhere from 10% to 100% of caregivers sought some form of health care for newborns with suspected illnesses, of which between 4% to 100% sought care from a trained medical provider and 4% to 66% specified receiving care at a secondary level health care facility. Despite these large variations in results, care seeking for newborn illnesses appears to be low in general and remains a key challenge to improving neonatal mortality.

Multiple factors may delay a caregiver's decision to seek care for their newborn, ranging from poor recognition of signs of illness, socio-cultural traditions regarding newborn seclusion, distance to a facility or provider, poor quality of care at facilities, lack of financial resources to access health care or transport, and the opportunity costs of missed work or childcare [35,38,39]. Yet there are effective strategies to address these barriers and increase the demand for newborn care. Innovative demand-side strategies have included community-based interventions to educate women in essential newborn care and birth preparedness, including illness recognition and timely, appropriate care seeking, and community mobilization initiatives to empower caregivers and communities to develop strategies to access care. Our review presents data from seven community-based intervention trials that primarily target essential newborn care, birth preparedness, and community mobilization via women's participatory groups, of which all showed an improvement of 4% to 30% in care seeking following the interventions. This finding supports similar findings from a recent meta-analysis of trials of community mobilization, where rates of institutional delivery increased by 70% in interventions groups, with a two-fold increase in studies with higher intensity mobilization activities [40]. Thus while baseline data on rates of neonatal care seeking are discouraging, there is promising evidence to suggest that innovative interventions can have a positive impact.

There is a need to simultaneously identify and strengthen supply-side strategies for neonatal health system development. However, merely increasing the availability of services, such as constructing more facilities or expanding health programs, may not directly result in an increase in the use of services. There is a role for combined strategies to increase demand for and supply of newborn health services [41]. For example, four intervention studies have explored the role of care acceptance [37,42–44]. In these South Asian trials, recognition and home-management of neonatal illness by trained health care workers has lead to an increase in care acceptance and a reduction of neonatal mortality. While these studies were not included in our review as they studied care acceptance, and not care-seeking decisions, they suggest home-based neonatal care is an acceptable and feasible intervention to enhance neonatal access to appropriate health care.

While neonatal care-seeking studies need to explore the extent to which factors delay and prevent care seeking, our review recognizes the inherent complexity of constructing a neonatal care-seeking framework. Our review found that a standardized and consistent approach to care-seeking behaviors is lacking, as illustrated by the absence of universal definitions and terminology in the reviewed studies. Definitions for illness varied widely, ranging from one observed symptom to the application of multilayered algorithms. Four studies failed to provide criterion on how neonatal illness was defined, identified, or established. Care-seeking definitions varied among studies, ranging from the inclusion of any care sought, including care provided at the home, care sought from a medically trained health care provider, or at a formally established health care facility. While some studies used the term "health care providers" to include traditional healers or village doctors, others applied it only to medically qualified providers and some studies did not distinguish

Table 4. Community-based studies that describe type of care sought for ill or suspected ill neonates.

Primary Author, Year	Neonates with Illness/Suspected Illness (*n*)	*n* Type of Care Sought (%)[a]						
		Health Care Facility			**Health Care Provider**		**Home Care**	**No Care**
		Secondary Health Center	**Primary Health Center**	**Pharmacy**	**Medically Trained Provider**	**Unqualified Provider**		
Community households surveys								
South Asia								
Ahmed, 2001 [55]	740	37 (4%)			89 (12%)	607 (82%)		96 (13%)
Bhandari, 2002 [56]	9	2 (22%)						
Mohan, 2008	202		24 (12%)		8 (4%)	12 (12%)		139 (69%)
Baqui, 2008 [58]	1226				498 (41%)			
Dongre, 2008 [59]	321	56 (17%)						
Dongre, 2009 [60]	27				27 (100%)	1 (1%)		
Willis, 2009 [61]	158				62 (39%)		58 (37%)	
Chowdhury, 2011 [62]	365			2 (1%)	136 (37%)	90 (25%)		137 (38%)
Africa region								
Bazzano, 2008 [63]	59				23 (39%)	13 (22%)		23 (39%)
Manji, 2009 [31]	N/A		N/A (83%)			N/A (1%)	N/A (16%)	
Waiswa, 2010 [64]	6	4 (66%)		2 (33%)				
Community-based intervention trials								
South Asia								
Manandhar, 2004 [26]	**C: 1320**; I: 919	**C: 131 (10%)**; I: 219 (24%)						
Kumar, 2008 [28]	**C: 296**; ENC: 319; ENCT: 227				**C: 49 (17%)**; ENC: 78 (25%); ENCT: 76 (33%)	**C: 186 (63%)**; ENC: 152 (48%); ENCT: 106 (47%)	**C: 19 (6%)**; ENC: 27 (9%); ENCT: 22 (10%)	
Dongre, 2009 [65]	**Before: 246; After: 147**	**Before: 119 (48%)**; After: 114 (78%)						
Darmstadt, 2010 [29]	C Base: 812; C End: 400; I Base: 733; I End: 1050				**C Base: 222 (27%)**; C End: 138 (35%); I Base: 225 (31%); I End: 198 (56%)			

[a]Percentage reported as a proportion of all neonates with an illness or suspected illness that were included in each study. Multiple responses regarding type of care sought were permitted, as described by included studies.
Abbreviations: C, control; ENC, essential newborn care; ENCT, essential newborn care plus thermostat; I, intervention; N/A, not available.
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between these provider types. While this lack of consistency is not a new occurrence, as noted by English et al. and the WHO Multicenter Study of Severe Infection in Young Infants [4,45], common definitions of "suspected newborn infection" and "appropriate" care-seeking behaviors have yet to be established. By addressing these inconsistencies and establishing standardized terms to identify barriers to care, future studies may be able to better generalize the factors and delays that influence neonatal care seeking.

Our findings further highlight the lack of neonatal-specific data. While published neonatal studies are mainly from South Asia, many studies have explored the care-seeking behaviors of caregivers for children under five years of age [46–50]. While a direct comparison of neonatal and non-neonatal care-seeking behaviors has not been studied, hospitalization rate comparisons have been published. Baqui et al., for example, found the hospitalization rate for acute lower respiratory infection in rural Bangladesh was nearly four-fold higher for children aged 1 to 5 mo than for neonates (108.9 versus 28.3 per 1,000 children-years observed) and recommended further research to identify reasons for the low hospital care usage [51]. Such findings suggest care-seeking behaviors among caregivers differ for neonates, infants, and children under five. Estimates for populations other than neonates may not be accurate or appropriate to use to approximate neonatal care-seeking patterns; this highlights the need to revise age categories used by ongoing databases and surveys, such as DHS, to specifically identify neonates and appropriately describe global and regional care-seeking behaviors.

While the study search was not limited by year, all studies that met inclusion criteria were published within the last decade, of which only four were not published within the last 5 y. Additionally, our search identified three publications that described neonatal care-seeking studies currently underway in Nepal, Malawi, and

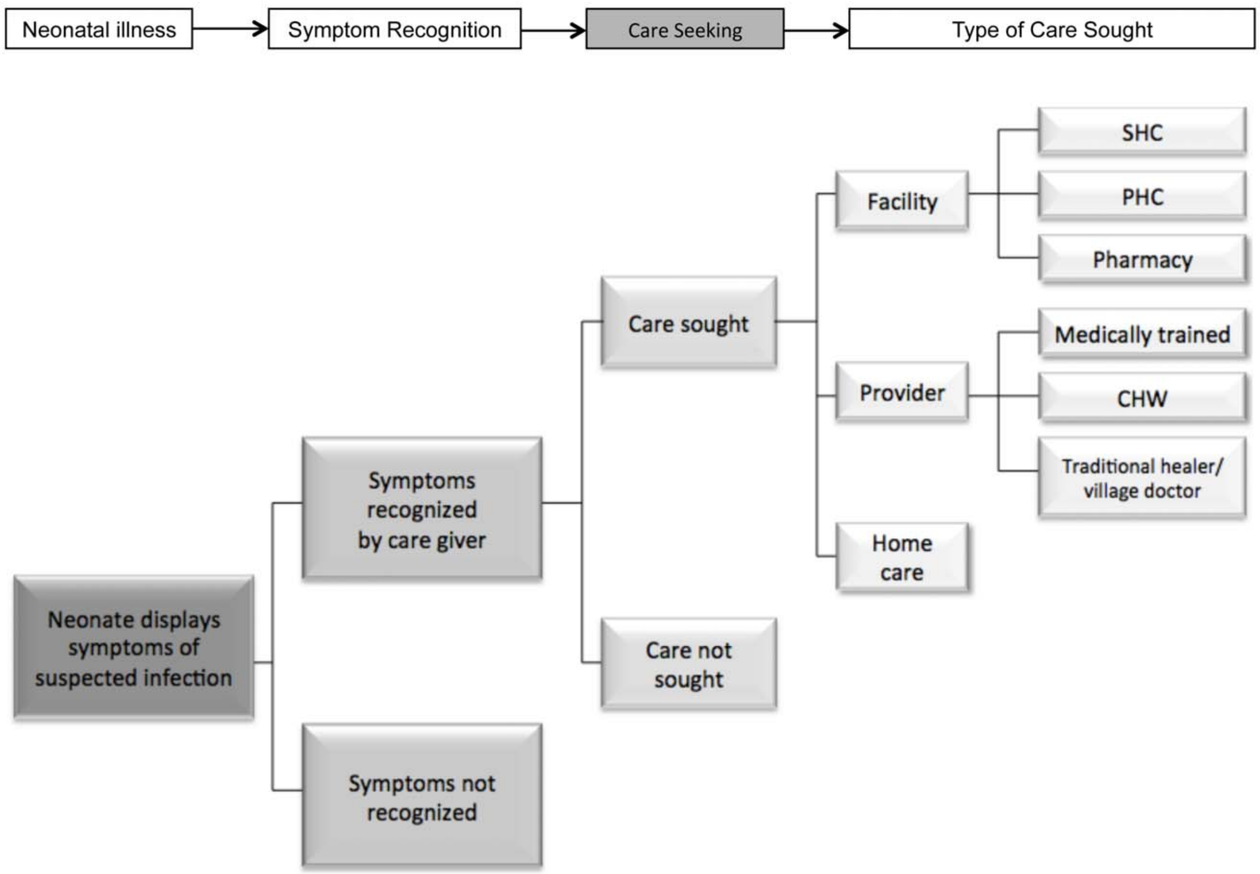

Figure 2. Defining care seeking conceptually. CHW, community health worker; PHC, primary health care; SHC, secondary health care.
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India [52–54]. Given the recent rise in care-seeking publications and the lack of data available before 2000, it is not possible to estimate whether care seeking for neonatal illness has improved with time. As care seeking continues to receive increasing attention within the literature, even more emphasis within the global arena is required to capture regional and time trends.

To bring about sustainable improvements in neonatal survival, changes are needed to both increase the demand for newborn care and strengthen health care systems, such as improving access and quality of care and socio-economic inequality through education and literacy initiatives. As shown in this review, there is a role for interventions within the community to encourage appropriate and timely care seeking. Community interventions provide important insight to identify and target barriers to care seeking. This paper serves as a call to action to enhance research efforts through the establishment of standardized definitions regarding illness criteria and care seeking and the classification of neonates as a specific age category in existing global databases. Additionally, community intervention research initiatives must be supported not only in South Asia, but throughout all regions of the world. By increasing global research efforts to define, understand, and address care seeking, we can continue to reduce the global burden of neonatal mortality.

Supporting Information

Text S1 PRISMA checklist.
(DOC)

Text S2 Study protocol.
(DOCX)

Author Contributions

Analyzed the data: HKH AC ACL. Wrote the first draft of the manuscript: HKH. Contributed to the writing of the manuscript: HKH AC ACL AB IR. ICMJE criteria for authorship read and met: HKH AC ACL AB IR. Agree with manuscript results and conclusions: HKH AC ACL AB IR.

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Editors' Summary

Background. Worldwide around 3.3 million babies die within their first month of life every year. While the global neonatal mortality rate has declined by 28% between 1990 and 2009 (from 33.2 deaths per 1,000 livebirths to 23.9), the proportion of under-five child deaths that are now in the neonatal period has increased in all regions of the world and currently stands at 41%. Of these deaths, over 90% occur in low- and middle-income countries (LMICs), making the risk of death in the neonatal period in LMICs more than six times higher than in high-income countries. In LMIC settings most babies are born at home so inappropriate and delayed care seeking can contribute substantially to neonatal mortality. Infection causes over a quarter of all deaths in neonates, but in LMICs diagnosis is often based on nonspecific clinical signs, which may delay the provision of care.

Why Was This Study Done? In order to improve neonatal survival in LMICs, health care facilities and providers must not only be available and accessible but a baby's caregiver, often a parent or other family member, must also recognize that the baby is ill and seek help. To address this problem with effective strategies, an understanding is needed of the patterns of care-seeking behavior by babies' caregivers in seeking help from health-care facilities or providers. In this study, the researchers explored the extent and nature of care-seeking behaviors by the caregivers of ill babies in LMIC settings.

What Did the Researchers Do and Find? Using multiple databases, the researchers conducted a comprehensive review up until October 2011 of all relevant studies including those that had not been formally published. Using specific criteria, the researchers then identified 22 appropriate studies (out of a possible 784) and recorded the same information from each study, including the number of neonates with illness or suspected illness, the number of care providers who sought care, and where care was sought. They also assessed the quality of each included study (the majority of which were from rural areas in South Asia) on the basis of a validated method for reviewing intervention effectiveness. The researchers found that the definitions of neonatal illness and care-seeking behavior varied considerably between studies or were not defined at all. Because of these inherent study differences it was not possible to statistically combine the results from the identified studies using a technique called meta-analysis, instead the researchers reported literature estimates and described their findings narratively.

The researchers' analysis included 9,098 neonates who were identified in community-based studies as being ill or suspected of being ill and a total of 4,320 related care-seeking events: care seeking ranged between 10% and 100% among caregivers including seeking care from a health facility (370) or from a health provider (1,813). Furthermore, between 4% to 100% of caregivers sought care from a trained medical provider and 4% to 48% specified receiving care at a health care facility: caregivers typically sought help from primary health care, secondary health care, and pharmacies and some from an unqualified health provider. The researchers also identified seven community-based intervention studies that included interventions such as essential newborn care, birth preparedness, and illness recognition, where all showed an increase in care seeking following the intervention.

What Do These Findings Mean? These findings highlight the lack of a standardized and consistent approach to neonate care-seeking behaviors described in the literature. However, despite the large variations of results, care seeking for newborn illnesses in LMICs appears to be low in general and remains a key challenge to improving neonatal mortality. Global research efforts to define, understand, and address care seeking, may help to reduce the global burden of neonatal mortality. However, to achieve sustainable improvements in neonatal survival, changes are needed to both increase the demand for newborn care and strengthen health care systems to improve access to, and quality of, care. This review also shows that there is a role for interventions within the community to encourage appropriate and timely care seeking. Finally, by addressing the inconsistencies and establishing standardized terms to identify barriers to care, future studies may be able to better generalize the factors and delays that influence neonatal care seeking.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.1001183.

- A recent *PLoS Medicine* study has the latest figures on neonatal mortality worldwide
- UNICEF provides information about progress toward United Nations Millennium Development Goal 4
- UNICEF also has information about neonatal mortality
- The United Nations Population Fund has information on home births

PLOS ONE

RESEARCH ARTICLE

Systematic review and meta-analysis of cohort studies of long term outdoor nitrogen dioxide exposure and mortality

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Abstract

Objective

To determine whether long term exposure to outdoor nitrogen dioxide (NO_2) is associated with all-cause or cause-specific mortality.

Methods

MEDLINE, Embase, CENTRAL, Global Health and Toxline databases were searched using terms developed by a librarian. Screening, data extraction and risk of bias assessment were completed independently by two reviewers. Conflicts were resolved through consensus and/or involvement of a third reviewer. Pooling of results across studies was conducted using random effects models, heterogeneity among included studies was assessed using Cochran's Q and I^2 measures, and sources of heterogeneity were evaluated using meta-regression. Sensitivity of pooled estimates to individual studies was examined and publication bias was evaluated using Funnel plots, Begg's and Egger's tests, and trim and fill.

Results

Seventy-nine studies based on 47 cohorts, plus one set of pooled analyses of multiple European cohorts, met inclusion criteria. There was a consistently high degree of heterogeneity. After excluding studies with probably high or high risk of bias in the confounding domain (n = 12), pooled hazard ratios (HR) indicated that long term exposure to NO_2 was significantly

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associated with mortality from all/ natural causes (pooled HR 1.047, 95% confidence interval (CI), 1.023–1.072 per 10 ppb), cardiovascular disease (pooled HR 1.058, 95%CI 1.026–1.091), lung cancer (pooled HR 1.083, 95%CI 1.041–1.126), respiratory disease (pooled HR 1.062, 95%CI1.035–1.089), and ischemic heart disease (pooled HR 1.111, 95%CI 1.079–1.144). Pooled estimates based on multi-pollutant models were consistently smaller than those from single pollutant models and mostly non-significant.

Conclusions

For all causes of death other than cerebrovascular disease, the overall quality of the evidence is moderate, and the strength of evidence is limited, while for cerebrovascular disease, overall quality is low and strength of evidence is inadequate. Important uncertainties remain, including potential confounding by co-pollutants or other concomitant exposures, and limited supporting mechanistic evidence. (PROSPERO registration number CRD42018084497)

Introduction

Traditional cohort studies involving recruitment of individual participants and long term follow-up over many years have been foundational in linking long term air pollution exposure to mortality [1, 2]. However, these studies are time-consuming and expensive, and may not be representative of the general population depending on recruitment procedures and response rates. More recently, large "synthetic" cohorts have been established by linking administrative or health survey data with address and mortality data [3–5]. Compared to traditional cohort studies, these newer cohort studies have the advantages of being less expensive and time-consuming, more statistically powerful and more representative of the general population. However, they potentially lack data on important covariates such as smoking, which could confound the association between air pollution and mortality. Both types of cohort studies have benefited from innovations in exposure assessment, including utilization of remote sensing, chemical/meteorological and dispersion models, as well as land use regression models [6–8]. These innovations have revolutionized exposure assessment, reducing reliance on sparse networks of ground monitors and increasing both geographic coverage and spatial resolution of estimated exposures.

With increasing exposure assessment capabilities, interest has grown in specific air pollution sources, including traffic. Nitrogen dioxide (NO_2) is a commonly employed marker of traffic-related urban air pollution [9, 10], although it also more broadly reflects any combustion in air, from sources such as industry and fossil fuel powered electric power generating stations [11, 12]. Ambient concentrations of NO_2 have declined considerably over the last 15–20 years in North America, Europe, Japan and South Korea, but concentrations are increasing in other areas (e.g. China, North Korea and Taiwan) [13]. Numerous studies have evaluated health effects of NO_2 on diverse body systems. However, whether long term NO_2 exposure is causally related to mortality remains an unresolved question. A particular complicating factor is whether NO_2 itself is to blame, or whether it is simply acting as a marker for specific air pollution sources i.e. emissions from vehicles, or more generally as a marker for spatial variation in the urban air pollution mixture [9]. Carbon monoxide and certain chemical components of fine particulate matter, also primarily originating from vehicle emissions, are key potential confounders, given their well-established pathophysiological mechanisms of action [14].

Effects of NO_2 could also be confounded by other concomitant traffic-related exposures such as noise or stress [15].

The aforementioned combination of methodological developments has led to rapid growth in the number and geographic diversity of cohort studies, and makes it timely to synthesize the available evidence. Five previous systematic reviews/ meta-analyses have evaluated the association of long term NO_2 exposure and mortality [16–20]. However, only one of these reviews conducted an assessment of risk of bias over multiple domains, none provided pooled estimates from paired single and multi-pollutant models from the same primary studies, and only studies published up to 2018 were included. Our objective is therefore to determine whether long term exposure to outdoor NO_2 is associated with all-cause or cause-specific mortality based on an up to date synthesis of the available evidence.

Methods

The protocol is registered with PROSPERO (CRD42018084497) (S1 File) [21].

Literature searches

MEDLINE, Embase, CENTRAL, Global Health and Toxline databases were searched using terms developed by a librarian (S1 Table). The search strategy underwent Peer Review of Electronic Search Strategies (PRESS) [22]. Searches were last updated February 25, 2020. Inclusion criteria were as follows: Participants/population: Humans; Intervention(s), exposure(s): Exposure to outdoor NO_2 (and other oxides of nitrogen); Comparator(s)/control: Lower levels of exposure; Main outcomes: Mortality from all/ natural causes or specific causes; Study design: cohort. Publications in abstract form only were excluded. Publications in English or French were included and there were no restrictions on publication date. Effect measures considered were: mortality effects reported as regression coefficients, hazard ratios (HR) or relative risks associated with exposures over a period of years, expressed per specified increment in exposure. The present review is one part of a series of reviews of effects of NO_2, all of which were included in the original search. Other reviews pertain to non-asthma respiratory morbidity and ischemic heart disease morbidity related to short term exposure [21]. Studies were selected for the present review if reported outcomes matched the inclusion criteria specified above.

Screening, data extraction and risk of bias assessment

Screening and data extraction were completed independently by two reviewers in DistillerSR. Conflicts between reviewers were resolved through consensus and/or involvement of a third reviewer. All studies retrieved from literature searches were screened for relevance based on title and abstract according to the above inclusion criteria. Where relevance could not be determined based on abstract and title, the full text was reviewed. Manual searches were also completed of reference lists of all relevant studies. Bibliographic data, study location and timing, population age group(s), sample size, cause of death (including the International Classification of Diseases (ICD) code(s) if available), method of exposure assessment, pollutant (including name, units, descriptive statistics), type of regression model, effect measure and standard error or confidence interval, model covariates (potential confounders) and their specification were extracted from all studies meeting inclusion criteria. When single pollutant results were presented for multiple exposure periods, we extracted the most highly statistically significant result (regardless of the direction of the association), or that reported by the authors as their primary finding. Results from multi-pollutant models that resulted in the greatest reduction in magnitude of effect compared to single pollutant results were selected in order to bracket the magnitude of effect from each study. Our objective in this regard was not to assess the magnitude of the association with NO_2 in the presence of a single co-pollutant common to all studies, but rather to determine the maximum potential for confounding of the association of mortality with NO_2 by any co-pollutant(s). Results expressed per pollutant increment expressed in μg/m^3 were converted to parts per billion [23], and those based on nitrogen oxides (NO_x) were converted by multiplying the log(HR) by 2.31 (the average ratio of log(HR) based on NO_2 to log(HR) based on NO_x in four studies based on 25 cohorts [24–27]). Where required data were not provided, authors were contacted by e-mail. In some instances Engauge Digitizer [28] was employed to extract numeric results presented only in graph form. The Navigation Guide systematic review methodology [29] was employed to evaluate risk of bias at the study level according to the following domains: selection bias, exposure assessment, confounding, outcome assessment, completeness of outcome data, selective outcome reporting, conflict of interest (including sources of funding) and other sources of bias. Criteria are detailed in S1 Fig. In the confounding domain, we considered age, sex, smoking and socioeconomic status as critical potential confounders. As a sensitivity analysis, we treated body mass index (BMI) rather than smoking as a critical confounder, as specified in the World Health Organization (WHO) risk of bias criteria [30]. Assessment of risk of bias was completed independently by two reviewers and conflicts between reviewers were resolved through consensus and/or involvement of a third reviewer.

Data analysis

Pooling of HRs across studies was conducted using random effects models computed using Restricted Maximum Likelihood (REML) estimation, with sensitivity analyses employing Dersimonian and Laird and Empirical Bayes estimators [31]. Heterogeneity among included studies was assessed using Cochran's Q and I^2 measures, and sources of heterogeneity were evaluated using meta-regression [31]. Sensitivity of pooled estimates to individual studies was examined using Leave One Out analysis and publication bias was evaluated using Funnel plots, Begg's and Egger's tests, and trim and fill [31]. Subgroup analyses were conducted by cause of death, region, risk of bias characterization (pre-specified in protocol) and single vs. multi-pollutant models. Analysis was conducted in R version 3.6.0 [32] using the metafor package [31].

Overall rating of quality and strength of evidence

We applied the Navigation Guide methodology [33] and the causality determination framework used by the US EPA and Health Canada [12] (S2 and S3 Tables) to judge the overall quality and strength of the evidence, and likelihood of a causal relationship. Following the Navigation Guide methodology, given the observational nature of the evidence, the starting point for rating overall quality was "moderate," which was downgraded or upgraded to "low" or "high" based on the criteria summarized in S2 Table [33]. Identification of multiple upgrading or downgrading factors does not necessarily mean that overall quality is upgraded or downgraded multiple levels. Rather, the overall degree of upgrading or downgrading is based on reviewer judgement of the overall importance and impact of all factors considered [33]. The Navigation Guide characterizes strength of evidence of toxicity as "sufficient", "limited", "inadequate" or "indicative of a lack of toxicity" (S2 Table), based on the overall quality of the evidence, the direction of effect, confidence in the effect and any other factors identified as germane by the reviewers [33]. Given the parallels with the USEPA/Health Canada causality determination criteria (S3 Table), while we did not conduct a systematic review of other lines of evidence, we drew upon summaries of other lines of evidence from a recent assessment document [11], supplemented by findings from more recent mechanistic studies, in order to characterize the likelihood of a causal relationship.

Results

A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram summarizing disposition of studies identified in literature searches is shown in Fig 1. As indicated earlier, the present review is one part of a series of reviews of effects of NO_2 on multiple outcomes, all of which were included in the original search, which is reflected in numeric results reported in Fig 1. Seventy-nine studies were included in our final analysis based on 47 cohorts, plus one set of pooled analyses of multiple European cohorts. Study characteristics are summarized in S4 Table. Approximately the same proportion of studies was conducted in North America (n = 32, 41%) and Europe (n = 35, 44%), while a smaller proportion (n = 12, 15%) was conducted elsewhere. Cohorts were mostly drawn from the general population, with the exception of six cohorts based on mortality follow-up after diagnosis of hypertension [34–38], myocardial infarction [39–41], stroke [42, 43], or lung cancer [26, 44]. Cohort sizes ranged

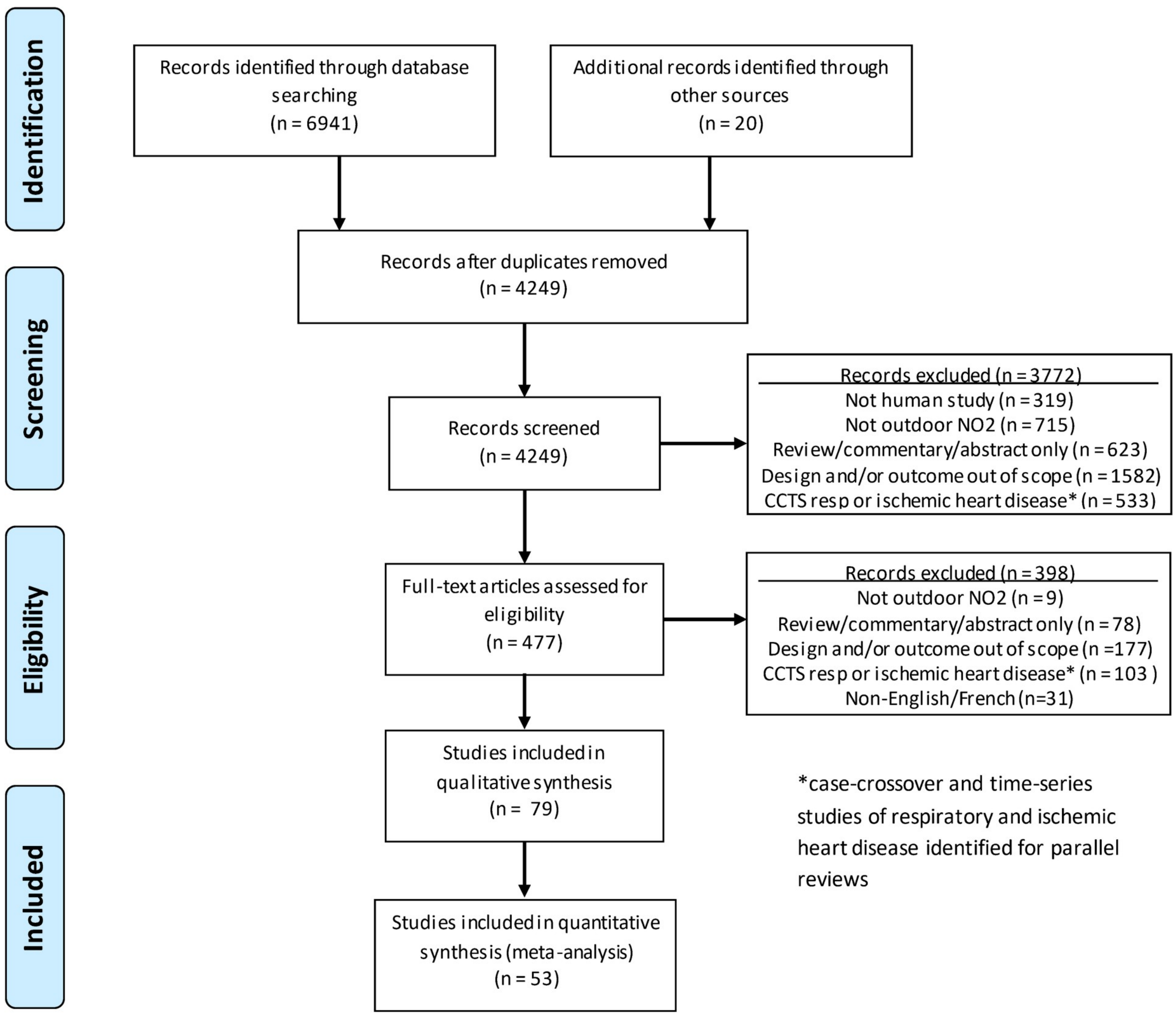

Fig 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

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from 1,800 to 14.1 million participants. Fifty-one studies (65%) employed modelling while 28 (35%) employed ground monitoring as the source of exposure data, and almost all, n = 74 (94%), employed NO_2 as the exposure metric, while only 5 (6%) employed NO_x. The most frequently examined causes of death were all/natural cause (non-accidental), (n = 56, 71%), cardiovascular (n = 37, 47%), respiratory (n = 35, 44%), lung cancer (n = 32, 41%), ischemic heart disease (n = 27, 34%), and cerebrovascular (n = 26, 33%). Thirty-eight studies (48%) were mostly conducted post 2000 (majority of study duration after 2000).

Risk of bias ratings for individual studies are shown in S2 Fig and reasons for assigned ratings of risk of bias greater than low risk (or unable to assess) for individual studies are provided in S5 Table. The greatest variability in ratings occurred in the exposure assessment and confounding domains, while ratings in the other six domains (selection bias, outcome assessment, completeness of outcome data, selective outcome reporting, conflict of interest, other sources of bias) were generally low or probably low risk of bias. Forty-six studies (58.2%) were rated probably high or high risk of bias or unable to assess in the exposure assessment domain (although bias would be expected to be non-differential) because exposure was based on an area-level average, did not account for change of address, relied on a single monitor, there was evidence of a mediocre correlation of modelled or measured values with ground measurements in the target community, or there was insufficient information. Twelve studies (15.2%) were rated probably high or high risk of bias in the confounding domain because of lack of adjustment for one or more key potential confounders (age, sex, smoking, socio-economic status). An additional eight studies did not adjust for BMI or account for obesity as a potential confounder through other covariates such as comorbidity or analysis of associations with exposure in complementary data.

Effect estimates and pooled effect estimates

All effect estimates from individual studies, including from single and multi-pollutant models, and by population and outcome subgroup are provided in forest plots by region in S3 Fig. Of these, we excluded estimates from pooling if they were superseded by other studies encompassing the same geographic area or time period, e.g. in subsequent multi-city studies or those spanning a longer study duration, results were provided only from multi-pollutant models, or there were too few studies of the outcome, leaving 53 studies included in the meta-analysis (see S4 Table). Pooled estimates based on single pollutant model results by cause of death are summarized in Table 1. Pooled estimates were largest for respiratory and cerebrovascular causes of death, similar for cardiovascular and ischemic heart disease, and smallest for all/natural cause and lung cancer. All pooled estimates were characterized by a high degree of heterogeneity.

Meta-regression and sensitivity analysis

Meta-regression revealed that the magnitude of the log HR for cardiovascular, respiratory and ischemic heart disease mortality was significantly larger for studies conducted outside North America and Europe (p = 0.002, 0.025, 0.021 respectively). For lung cancer mortality, standard deviation (p = 0.0009) and range (p = 0.006) of NO_2 exposure were significantly positively associated with log HR. Probably high or high risk of bias in the confounding domain was significantly positively associated with log HR for all/ natural cause (p = 0.040), respiratory (p = 0.006) and cerebrovascular (p = 0.036) mortality. In a sensitivity analysis, treating BMI rather than smoking as a critical potential confounder, as specified in the WHO risk of bias criteria, probably high or high risk of bias in the confounding domain was not associated with log HR. Risk of bias in the exposure assessment domain, study interquartile range NO_2 and timing of study primarily before or after 2000 were not significant predictors of the log HR. Residual

Table 1. Pooled estimates by cause of death and sensitivity analyses.

Cause of death	N	P(Q)	I^2 (%)	Pooled HR per 10 ppb	Lower 95% CI	Upper 95% CI
All/ Natural causes	39	<0.0001	99.3	1.064	1.034	1.094
Cardiovascular	29	<0.0001	99.9	1.139	0.997	1.301
Respiratory	29	<0.0001	99.7	1.174	1.016	1.357
Lung cancer	28	<0.0001	96.6	1.084	1.045	1.124
Ischemic heart disease	19	<0.0001	96.1	1.128	1.076	1.182
Cerebrovascular	17	<0.0001	99.7	1.167	0.936	1.456
Excluding studies with probably high or high risk of bias in confounding domain						
All/ Natural causes	32	<0.0001	96.7	1.047	1.023	1.072
Cardiovascular	23	<0.0001	92.8	1.058	1.026	1.091
Respiratory	24	<0.0001	65.9	1.062	1.035	1.089
Lung cancer	23	<0.0001	85.1	1.083	1.041	1.126
Ischemic heart disease	14	0.0001	69.9	1.111	1.079	1.144
Cerebrovascular	13	0.0738	0.1	1.014	0.997	1.032
Excluding studies with probably high or high risk of bias in confounding domain and exposure based on NO_x						
All/ Natural causes	29	<0.0001	92.4	1.033	1.016	1.051
Cardiovascular	21	<0.0001	94.3	1.056	1.020	1.093
Respiratory	21	<0.0001	67.6	1.057	1.031	1.085
Lung cancer	21	<0.0001	85.6	1.075	1.033	1.119
Ischemic heart disease	12	0.0003	56.7	1.104	1.078	1.131
Cerebrovascular	11	0.0488	0.5	1.013	0.996	1.032
Excluding studies with probably high or high risk of bias in confounding domain (WHO criteria–BMI rather than smoking as critical potential confounder)						
All/ Natural causes	31	<0.0001	98.4	1.063	1.027	1.100
Cardiovascular	19	<0.0001	98.1	1.097	1.025	1.173
Respiratory	18	0.0129	0.1	1.041	1.028	1.054
Lung cancer	17	<0.0001	89.7	1.082	1.0279	1.134
Ischemic heart disease	14	<0.0001	91.4	1.149	1.093	1.208
Cerebrovascular	13	<0.0001	96.3	1.080	0.973	1.200

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heterogeneity remained high (I^2 >80%) even after accounting for significant predictor variables.

Based on meta-regression findings, studies with probably high or high risk of bias in the confounding domain (n = 12) were excluded, resulting in substantially smaller pooled HRs for cardiovascular, respiratory and cerebrovascular mortality (Table 1). Pooled estimates were significantly smaller for cerebrovascular mortality (p = 0.031) and significantly larger for ischemic heart disease mortality (p = 0.002), than for all/ natural cause mortality. Fig 2 presents a forest plot of HRs from individual studies and pooled estimates for all/ natural cause mortality, after exclusion of studies with probably high or high risk of bias in the confounding domain. The pooled estimate for North America was smaller than those for Europe and other countries, and heterogeneity among primary studies was lower for North American studies. Forest plots for other causes of death are provided in S4 Fig. Applying the WHO risk of bias criteria (treating BMI rather than smoking as a critical potential confounder) resulted in a somewhat larger pooled estimate for all/natural cause mortality, considerably larger pooled estimates for cardiovascular and cerebrovascular mortality, together with much greater heterogeneity for the latter, and a somewhat smaller pooled estimate with narrower confidence intervals and considerably less heterogeneity for respiratory mortality (Table 1).

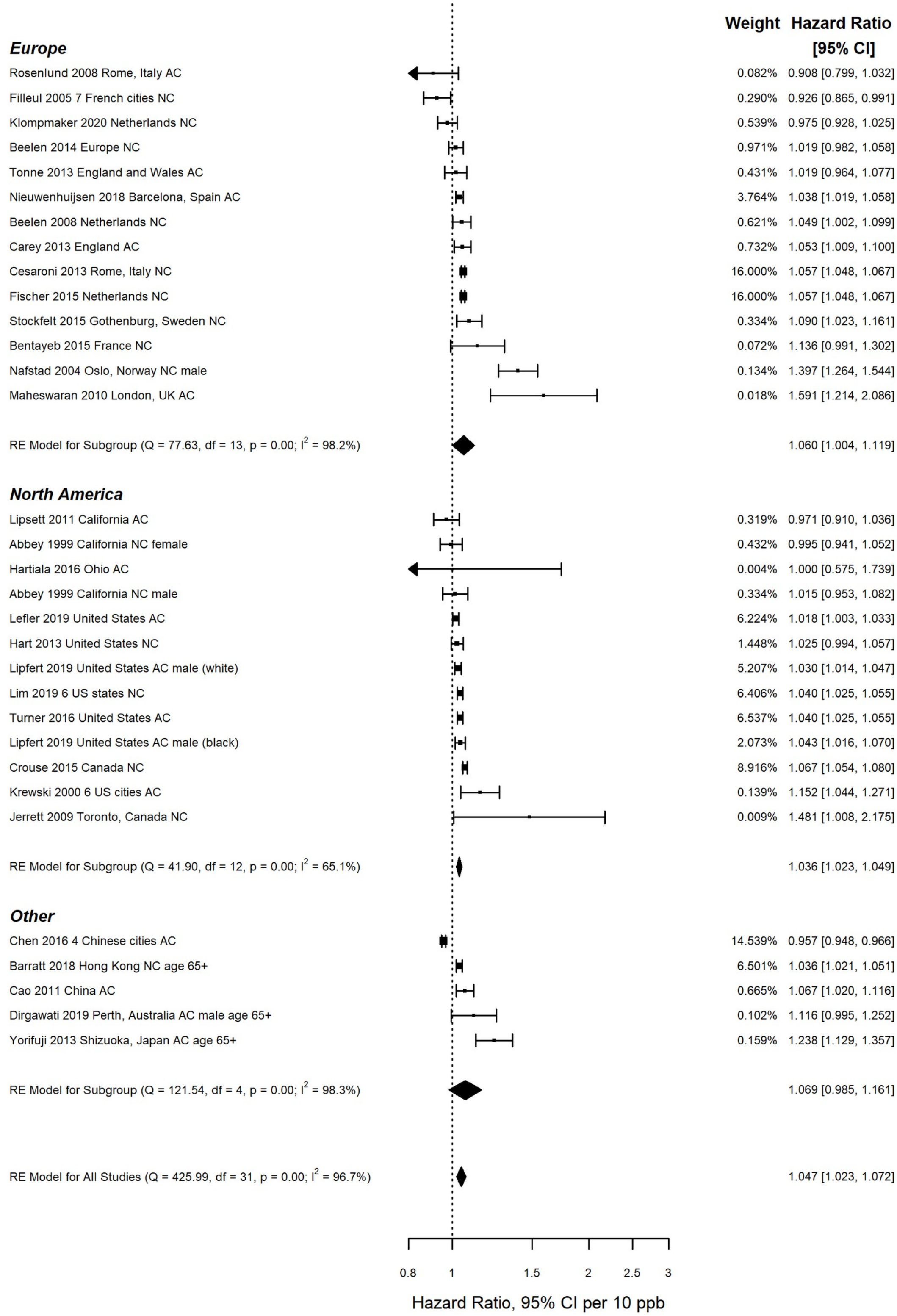

Fig 2. Hazard ratios from single pollutant models from individual cohort studies and pooled estimates by region, for all/ natural cause mortality (AC/NC).

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Results were generally insensitive to the exclusion of studies employing NO_x as the exposure metric [45–47], with the exception of all/natural cause mortality, for which the pooled estimate was somewhat smaller (Table 1). Findings were also insensitive to excluding cohorts of individuals with pre-existing disease [36, 39, 41, 42] (pooled estimate of HR for all/natural cause mortality 1.050, 95% CI 1.022–1.078). Pooled estimates were not sensitive to employing alternative estimators except that confidence intervals tended to be slightly narrower based on Dersimonian and Laird, and slightly wider based on Empirical Bayes (S1 Text). In leave one out analyses, heterogeneity among respiratory and ischemic heart disease studies was sensitive to exclusion of results from Katanoda et al. [48] and Turner et al. [49] respectively (S6 Table). Egger's test revealed significant funnel plot asymmetry for all/ natural cause ($p = 0.0022$) and lung cancer ($p = 0.031$) mortality, while Begg's test indicated significant funnel plot asymmetry only for lung cancer mortality ($p = 0.022$). Trim and fill analysis estimated that there were 4 missing studies with log HR below the pooled estimate for lung cancer mortality (S5 Fig), reducing the pooled estimate slightly to 1.066 (95%CI 1.024–1.110).

Effects of co-pollutants, noise and green space

A forest plot of nine paired estimates of associations with all/ natural cause mortality from single and multi-pollutant models from the same study, after exclusion of studies with probably high or high risk of bias in the confounding domain, is shown in Fig 3. The pooled estimate from single pollutant models was higher than that from multi-pollutant models with 1–4 co-

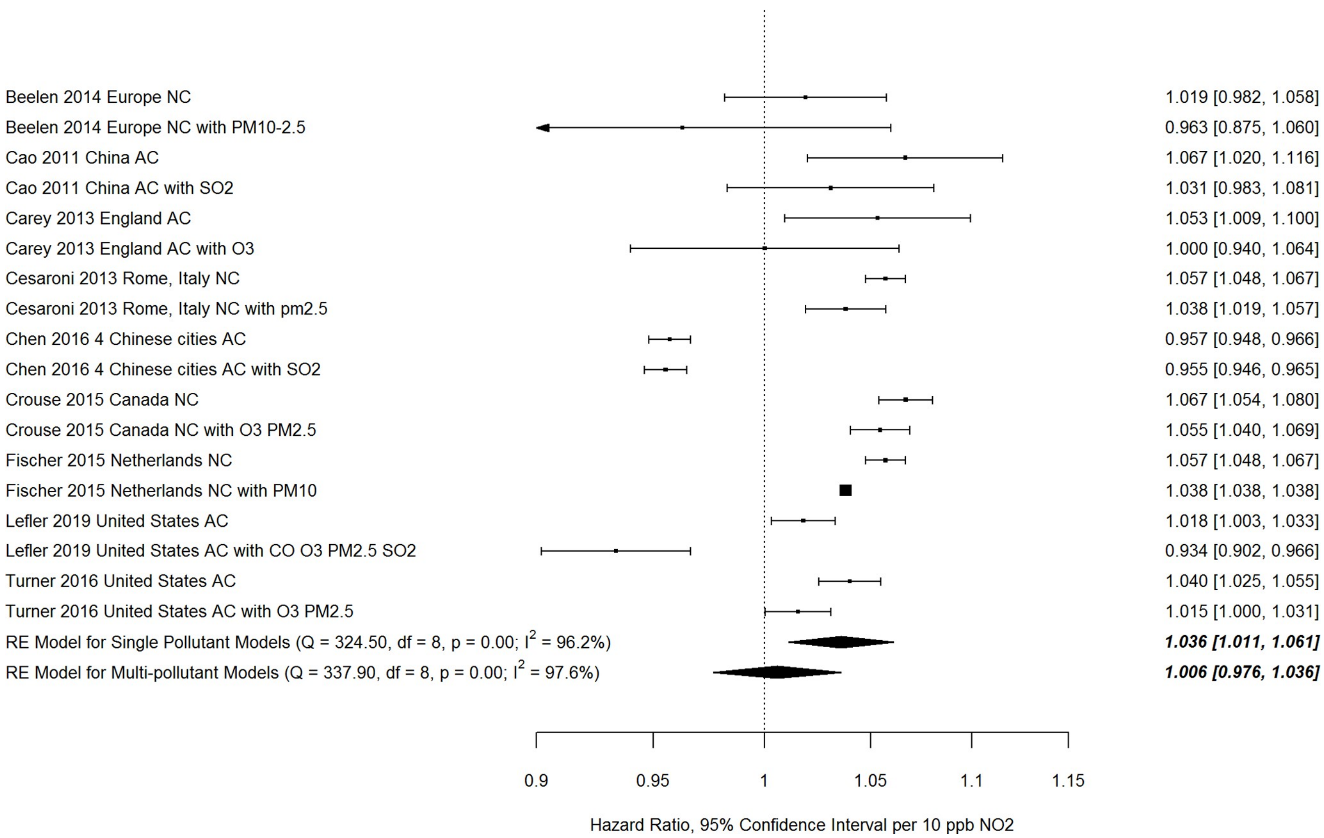

Fig 3. Hazard ratios from individual cohort studies and pooled estimates from single and multi-pollutant models for all/ natural cause mortality (AC/NC).

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pollutants, and the confidence interval for the multi-pollutant pooled estimate included 1, indicating no association. However, the difference between pooled estimates for single and multi-pollutant models was not significant (p = 0.13). Fewer paired estimates from single and multi-pollutant models were available for other causes of death. Pooled estimates from multi-pollutant models were consistently smaller in magnitude than those from single pollutant models, and the pooled estimates based on multi-pollutant models included 1, indicating no association, with the exception of ischemic heart disease mortality which was based on only three studies (Table 2).

Few studies have jointly examined effects of NO_2 and noise. Sørensen et al. found that NO_2 exhibited a significant positive association with fatal stroke when modelled jointly with traffic noise [50], while in the same cohort, Hvidtfeldt et al. found that NO_2 remained significantly associated with natural cause mortality after adjustment for traffic noise, although the magnitude of the association was reduced, and NO_2 was no longer significantly associated with cardiovascular mortality after adjustment for traffic noise [51]. Héritier et al. [52] also reported that the association of NO_2 with myocardial infarction mortality was no longer significant after adjustment for traffic, rail and aircraft noise. Klompmaker et al. found no association between NO_2, $PM_{2.5}$, greenness or traffic noise and mortality [53], and Tonne et al. observed a negligible change in the association between NO_2 and mortality with additional adjustment for traffic noise [40]. Nieuwenhuijsen et al. [54] found that the association of NO_2 with mortality was reduced in magnitude in models with noise and/or green space.

Shape of exposure-response relationship

Twenty-nine studies evaluated the shape of the exposure-response relationship between NO_2 or NO_x and mortality by examining the association by pollutant quantile [24, 41, 44, 47, 55–57], plotting the association using a non-linear function of pollutant concentration and/or testing the significance of the difference between linear and non-linear models [5, 25, 27, 45, 46, 50, 51, 53, 57–69], or plotting associations by city [48]. Of these, 19 studies found a linear association [27, 41, 44, 50, 51, 53, 55–57, 60, 63–65, 67–69], in some instances only in subsets of the data by cause of death [5, 45, 46], and five studies concluded that the association was supralinear [25, 45, 47, 59, 66]. Three found evidence of a threshold [48, 61, 62], but only one study identified specific numeric values, ranging from 20–40 $\mu g/m^3$, depending on age and cause of death [62]. Two studies reported no association, based on analysis by quantiles [24], and using a non-linear function of NO_2 [58]. While there was some variation in findings by cause of

Table 2. Pooled estimates from single and multi-pollutant models by cause of death.

Cause of death	Model	n	Pooled HR (95% CI)	p (difference)
All/natural cause	Single	9	1.036 (1.011–1.061)	0.13
	Multi	9	1.006 (0.976–1.036)	
Cardiovascular	Single	4	1.053 (1.011–1.096)	0.26
	Multi	4	1.018 (0.975–1.063)	
Lung cancer	Single	5	1.057 (0.967–1.156)	0.65
	Multi	5	1.028 (0.948–1.115)	
Respiratory	Single	5	1.039 (1.024–1.054)	0.21
	Multi	5	1.013 (0.977–1.051)	
Cerebrovascular	Single	2	1.000 (0.976–1.025)	0.67
	Multi	2	0.977 (0.879–1.086)	
Ischemic heart disease	Single	3	1.106 (1.064–1.150)	0.21
	Multi	3	1.071 (1.038–1.105)	

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death in individual studies, overall there was evidence of an exposure-response relationship across all six causes of death.

Discussion

Based on an analysis of 53 cohort studies, pooled estimates of associations indicated that long term exposure to outdoor NO_2 was significantly associated with mortality from all/ natural causes, cardiovascular disease, lung cancer, respiratory disease, and ischemic heart disease. There was a consistently high degree of heterogeneity among results from primary studies, which was only partially accounted for in meta-regression. The magnitude of the observed association was larger in studies with probably high or high risk of bias in the confounding domain, and pooled estimates were substantially smaller for cardiovascular, respiratory and cerebrovascular mortality when these studies were excluded. Heterogeneity based on I^2 was reduced substantially for cerebrovascular and respiratory mortality after excluding these studies, which we believe is related to the combination of a smaller number of studies and exclusion of studies reporting associations of a magnitude that differed substantially from the remaining studies. We also found that pooled estimates were sensitive to which covariates were considered critical potential confounders; pooled estimates for cardiovascular and cerebrovascular mortality were considerably larger when BMI rather than smoking was treated as a critical potential confounder, as specified in the WHO risk of bias criteria. We did not find an association between log HR and risk of bias in the exposure assessment domain, although one would expect that greater exposure measurement error (likely non-differential) would tend to bias associations towards the null, depending on the type of error [70–73]. Indeed, Crouse et al. reported that the magnitude of the association was larger when change of residential address was accounted for [59]. There was evidence of publication bias based on tests of funnel plot asymmetry for all/ natural cause and lung cancer mortality, but application of trim and fill revealed only small reductions in pooled estimates of association. The pooled estimate for all/natural cause mortality based on single pollutant models was larger than that based on multi-pollutant models, and confidence intervals on the multi-pollutant pooled estimate overlapped 1 or no association. Of the approximately one-third of studies that examined the shape of the exposure response relationship, most concluded that it was linear.

There have been five previous systematic reviews/meta-analyses of the association between long term NO_2 exposure and mortality in cohort studies. Pooled estimates from these studies are summarized in Table 3 in comparison with the present study, by cause of death. With the exception of the recent paper by Huangfu and Atkinson [20], for all four categories of cause of death, pooled estimates from the present study were based on approximately twice as many primary studies as the most recent previous systematic review. Pooled estimates varied most for cardiovascular and respiratory mortality, while they were relatively consistent in magnitude for all/natural cause and lung cancer mortality. Our pooled estimate was the largest for respiratory mortality based on all studies, but consistent with lower pooled estimates from other studies after excluding those with probably high or high risk of bias in the confounding domain. Heterogeneity among results from primary studies was uniformly high in earlier meta-analyses, with the exception of one pooled estimate for respiratory mortality based on only 8 primary studies. Only Huangfu and Atkinson [20], conducted an assessment of risk of bias over multiple domains, but neither they nor the other systematic reviews provided pooled estimates from paired single and multi-pollutant models from the same primary studies. In subgroup analyses, Atkinson et al. found that pooled estimates of associations with all/ natural cause, cardiovascular and lung cancer mortality based on cohorts restricted to older age groups were larger than those based on all ages [19]. Additionally, those based on models adjusting

Table 3. Comparison of pooled estimates from current study and previous meta-analyses.

Cause of death	Author	Central[b]	Lower 95%CI	Upper 95%CI	n	I^2 (%)
All/Natural cause	Stieb 2020	1.064	1.034	1.094	39	99
	Stieb 2020[a]	1.047	1.023	1.072	32	97
	Huangfu 2020	1.038	1.010	1.067	24	97
	Atkinson 2018	1.038	1.019	1.057	20	84
	Faustini 2014	1.079	1.036	1.124	12	89
	Hoek 2013	1.106	1.059	1.156	12	73
Cardiovascular	Stieb 2020	1.139	0.997	1.301	29	100
	Stieb 2020[a]	1.058	1.026	1.091	23	93
	Faustini 2014	1.265	1.172	1.365	16	98
	Atkinson 2018	1.057	1.038	1.096	15	83
Respiratory	Stieb 2020	1.174	1.016	1.357	29	100
	Stieb 2020[a]	1.062	1.035	1.089	24	66
	Huangfu 2020	1.057	1.019	1.097	15	83
	Atkinson 2018	1.057	1.019	1.096	13	76
	Faustini 2014	1.046	1.032	1.061	8	0
Lung cancer	Stieb 2020	1.084	1.045	1.124	28	97
	Stieb 2020[a]	1.083	1.041	1.126	23	85
	Atkinson 2018	1.096	1.038	1.156	16	88
	Hamra 2015	1.077	1.019	1.156	15	73

[a]After exclusion of studies with probably high or high risk of bias in confounding domain.

[b]Per 10 ppb.

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for key confounders (BMI, smoking) were smaller than those without these adjustments (natural cause, cardiovascular, respiratory and lung cancer mortality), similar to our findings; and those for natural cause and cardiovascular mortality based on residential exposure estimates were larger than those based on area level exposures [19]. In Faustini et al's systematic review, the effects of excluding studies based on individuals with pre-existing disease, older age groups, or with area level exposure characterization were inconsistent [17]. Hamra et al. found little impact of method of exposure characterization or presence or absence of adjustment for selected confounders (with the exception of studies including adjustment for occupation, which generated a smaller pooled estimate) [18]. Huangfu and Atkinson also found little impact of adjustment for selected confounders [20]. They concluded that the certainty of evidence was moderate for all-cause, respiratory and acute lower respiratory infection mortality, and high for chronic obstructive pulmonary disease mortality [20].

Other lines of evidence

The 2016 US Environmental Protection Agency (EPA) Integrated Science Assessment on oxides of nitrogen concluded that toxicological evidence suggested there were several possible mechanisms through which long term exposure to NO_2 could contribute to adverse respiratory and cardiovascular effects, including pulmonary inflammation, oxidative stress, pulmonary injury, changes in lung morphology, impaired respiratory immune defences, atherosclerosis, autonomic dysfunction, and changes in lipid metabolism [11]. Examples of findings from relevant studies include the modulation of alveolar macrophage activity in rats in association with NO_2 exposure [74], and increased mortality from respiratory infection in mice in two older studies of NO_2 exposure [75, 76]. With respect to cardiovascular effects, in one study, long term exposure to NO_2 was associated with reduced heart rate variability, but only in women [77]. In a Spanish cross sectional study, long term NO_2 exposure was associated with increased blood pressure [78] and subclinical atherosclerosis [79]. A more recent study in Germany found that those with higher long term NO_2 exposure had significantly higher odds of elevated ankle-brachial index, reflective of arterial stiffening, although the magnitude of the association was reduced in a joint model with $PM_{2.5}$ absorbance (a proxy for elemental carbon) [80]. Overall, however, the evidence base was considered limited, associations were inconsistent, and it was difficult to separate effects of NO_2 from those of co-pollutants [11]. With respect to short term exposure to NO_2 and mortality, a recent systematic review concluded that there was a high degree of certainty of the evidence linking 24 hour average exposure and mortality [81]. While there may be a degree of overlap in the effects captured by studies of short term and long term exposure, the overall health impact captured by each type of design is not identical [82], and certainty of evidence regarding effects of short term exposure does not necessarily imply certainty regarding effects of long term exposure.

Overall rating of quality and strength of evidence

In their 2016 Science Assessments, both the US EPA and Health Canada concluded that the evidence was suggestive of, but not sufficient to infer, a causal relationship between long term NO_2 exposure and mortality, based on a smaller number of studies, and fewer studies examining the impact of adjustment for co-pollutants than considered here, as well as limited and inconsistent supporting mechanistic evidence from human and animal studies [11, 12]. Applying the Navigation Guide methodology [33] and the causality determination framework used by the US EPA and Health Canada [12] to our current findings, several factors are considered downgrading factors in interpreting the overall quality of evidence. These include the significant heterogeneity among studies even after accounting for sources of heterogeneity, and the relatively large proportion of studies rated as probably high or high risk of exposure assessment bias (57.1%) (even though presence of this bias was not associated with magnitude of association in meta-regression). There was also evidence of publication bias, particularly for lung cancer mortality, but it did not have a substantial impact on pooled estimates of association. Risk of bias from residual confounding was evaluated both in relation to inclusion of critical potential confounders in statistical models, as well as impacts of co-pollutants and other co-exposures on the magnitude of associations. Pooled estimates indicated that NO_2 remained significantly associated with all/natural cause, cardiovascular, lung cancer, respiratory and ischemic heart disease mortality after exclusion of 12 studies with probably high or high risk of bias in the confounding domain. However, after excluding these studies, only 9 studies of all/natural cause mortality provided estimates based on both single and multi-pollutant models, and the pooled estimate indicated that NO_2 was no longer significantly associated with mortality after adjusting for co-pollutants. Fewer paired estimates from single and multi-pollutant models were available for other causes of death. Pooled estimates from multi-pollutant models were consistently smaller in magnitude than those from single pollutant models, and the pooled estimates based on multi-pollutant models included 1, indicating no association, with the exception of ischemic heart disease mortality, which was based on only three studies. Multi-pollutant models should be interpreted with caution in that the sensitivity of the effect of one pollutant to inclusion of other pollutants in a joint model is affected by factors such as the correlation among pollutants and their relative degree of exposure measurement error [83]. There is nonetheless evidence of confounding by co-pollutants of the association of long term NO_2 exposure with mortality. Few studies jointly modelled NO_2 with traffic noise. In a recent review, Tétrault et al. concluded that cardiovascular effects of long term air pollution exposure were probably independent of noise, but this was based on only nine studies, including only one study of air pollution and mortality [15]. One study found that that the association of NO_2 with mortality was reduced in magnitude in models with both noise and/or green space [54]. Specifically with respect to cerebrovascular mortality, imprecision is also considered a downgrading factor, as the 95% CI on the pooled estimate overlapped the null. In contrast to these downgrading factors, characterization of the exposure-response relationship as linear, supralinear, or linear with a threshold in 27 of the 29 studies in which this was evaluated, is considered an upgrading factor applicable to all six causes of death. Huangfu and Atkinson also downgraded the evidence in relation to heterogeneity for all cause and respiratory mortality, and upgraded it in relation to evidence of an exposure-response relationship [20]. Based on these considerations, we conclude that for all causes of death other than cerebrovascular disease, the overall quality of the evidence from cohort studies is moderate, the strength of evidence of toxicity is limited, and the overall evidence continues to be suggestive of, but not sufficient to infer, a causal relationship between long term NO_2 exposure and mortality. In the case of cerebrovascular disease mortality, owing to the smaller number of primary studies and the non-significant smaller magnitude association based on the pooled estimate, we conclude that the overall quality of the evidence from cohort studies is low, the strength of evidence is inadequate, and the overall evidence is inadequate to infer a causal relationship. Upgrading to a conclusion that there is sufficient evidence for a causal relationship would require more conclusive evidence ruling out potential confounders as well as consistent supporting animal toxicological and human clinical evidence. Future studies could address uncertainties related to confounding by co-pollutants by more consistently examining their correlations and effects in multi-pollutant models, in particular adjusting for other traffic-related pollutants and concomitant exposures like noise, green space and stress. Only about one third of the studies we reviewed addressed the shape of the concentration-response relationship, therefore examination of this issue in future studies would also be informative. While the evidence reviewed does not support the unequivocal conclusion that long term exposure to outdoor NO_2 causes an increased risk of death, identifying the true causal agent is of major importance to public health. Vehicle emissions are one of the main sources of NO_2, but vehicle emissions, and secondary pollutants arising from vehicle emissions also include numerous other potentially toxic pollutants such as carbon monoxide, particulate matter, benzene, formaldehyde, acetaldehyde, 1,3-butadiene, ozone, nitrates and organic and inorganic acids [10]. If the true causal agent is not NO_2, control measures which specifically reduce NO_2 will not reduce mortality risks. Conversely, identification and control of the true causal agent will have considerable public health benefits.

Conclusions

We conducted a synthesis of the evidence from 79 cohort studies examining the association between long term NO_2 exposure and natural cause and cause-specific mortality, including sensitivity analyses based on pooling method, leave one out analysis and trim and fill, meta-regression to examine sources of heterogeneity, and analysis of single vs. multi-pollutant models. We concluded that for all causes of death other than cerebrovascular disease, the overall quality of the evidence is moderate and the strength of evidence of toxicity was categorized as limited, while for cerebrovascular disease the overall quality of the evidence is low, and strength of evidence was rated inadequate. Important uncertainties remain, including potential confounding by co-pollutants or other concomitant exposures, and limited supporting mechanistic evidence. Identification and control of the true causal agent linking long term NO_2 exposure and mortality, whether NO_2 itself or another correlated exposure, will have considerable public health benefits.

Supporting information

S1 Fig. Risk of bias criteria.
(PDF)

S2 Fig. Risk of bias ratings for individual studies.
(PDF)

S3 Fig. Forest plots by region.
(PDF)

S4 Fig. Hazard ratios from single pollutant models from individual cohort studies and pooled estimates by region (specific causes of death).
(PDF)

S5 Fig. Funnel plot of log(hazard ratio) vs. standard error, lung cancer mortality.
(PDF)

S1 File. Review protocol.
(PDF)

S2 File. Data.
(CSV)

S3 File. PRISMA 2009 checklist.
(PDF)

S1 Table. Details of search strategies.
(PDF)

S2 Table. Navigation guide criteria for overall quality and strength of evidence.
(PDF)

S3 Table. USEPA/Health Canada criteria for evaluating likelihood of causal relationship.
(PDF)

S4 Table. Characteristics of primary studies.
(PDF)

S5 Table. Reasons for assigned ratings of risk of bias greater than low risk (or unable to assess).
(PDF)

S6 Table. Leave one out analyses.
(PDF)

S1 Text. Sensitivity to estimator.
(PDF)

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