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Anti-vascular endothelial growth factor (VEGF) drugs for treatment of retinopathy of prematurity (Review)

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T A B L E   O F   C O N T E N T S

[Intervention Review]

Anti-vascular endothelial growth factor (VEGF) drugs for treatment of retinopathy of prematurity

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A B S T R A C T

Background

Vascular endothelial growth factor (VEGF) plays a key role in angiogenesis in fetal life. Recently, researchers have attempted to use anti-VEGF agents for the treatment of retinopathy of prematurity (ROP), a vasoproliferative disorder. There is currently uncertainty regarding the safety and efficacy of these agents in preterm infants with ROP.

Objectives

To evaluate the efficacy and safety of anti-VEGF drugs when used either as monotherapy, i.e. without concomitant cryotherapy or laser therapy or in combination with planned cryo/laser therapy in preterm infants with type 1 ROP (defined as zone I any stage with plus disease, zone I stage 3 with or without plus disease or zone II stage 2 or 3 with plus disease).

Search methods

We searched the Cochrane Central Register of Controlled Trials (CENTRAL 2016, Issue 1), MEDLINE (1966 to January 1, 2016), EMBASE (1980 to January 1, 2016), CINAHL (1982 to January 1, 2016), conference proceedings, and previous reviews.

Selection criteria

Randomised or quasi-randomised controlled trials that evaluated the efficacy and safety of administration, or both, of anti-VEGF agents compared with conventional therapy in premature infants with ROP.

Data collection and analysis

We used standard Cochrane and Cochrane Neonatal methods for data collection and analysis.

Main results

Three trials, in which 239 infants participated, fulfilled the inclusion criteria. Two trials compared intravitreal bevacizumab with conventional laser therapy (monotherapy) while the third compared intravitreal pegaptanib *plus* laser treatment with laser and cryotherapy (combination therapy) in infants with type 1 ROP.

Of the two studies that evaluated intravitreal bevacizumab, one randomized infants while the other randomized eyes of the infants to the intervention and control groups. The former did not report any difference in the incidence of complete or partial retinal detachment between the groups (143 infants; RR 1.04, 95% CI 0.21 to 5.13; RD 0.00, 95% CI -0.06 to 0.07; very low quality evidence) but reported a significant reduction in the risk of refractive errors - very high myopia - at 30 months of age (211 eyes; RR 0.06, 95% CI 0.02 to 0.20; RD -0.40, 95% CI -0.50 to -0.30; low quality evidence) and recurrence of ROP by 54 weeks' postmenstrual age (143 infants; RR 0.22, 95% CI 0.08 to 0.62; RD -0.20, 95% CI -0.31 to -0.09; moderate quality evidence) in the bevacizumab group. The study found no difference in the risk of mortality before discharge from the hospital (150 infants; RR 1.50; 95% CI 0.26 to 8.75; RD 0.01; 95% CI -0.04 to 0.07; low quality evidence), mortality at 30 months of age (150 infants; RR 0.86, 95% CI 0.30 to 2.45; RD -0.01; 95% CI -0.10 to 0.08; low quality evidence), corneal opacity requiring corneal transplant (286 eyes; RR 0.34, 95% CI 0.01 to 8.26; RD -0.01; 95% CI -0.03 to 0.02; very low quality evidence), or lens opacity requiring cataract removal (286 eyes; RR 0.15, 95% CI 0.01 to 2.79; RD -0.02; 95% CI -0.05 to 0.01; very low quality evidence). The second trial that randomized eyes of the infants did not find any difference in the risk of complete retinal detachment between the eyes randomized to bevacizumab and those that were randomized to laser therapy (13 eyes; RR 0.33, 95% CI 0.01 to 7.50; RD -0.08, 95% CI -0.27 to 0.11).

When used in combination with laser therapy, intravitreal pegaptanib was found to reduce the risk of retinal detachment when compared to laser/cryotherapy alone (152 eyes; RR 0.26, 95% CI 0.12 to 0.55; RD -0.29, 95% CI -0.42 to -0.16; low quality evidence). The incidence of recurrence of ROP by 55 weeks' postmenstrual age was also lower in the pegaptanib + laser therapy group (76 infants; RR 0.29, 95% CI 0.12 to 0.7; RD -0.35, 95% CI -0.55 to -0.16; low quality evidence). There was no difference in the risk of perioperative retinal haemorrhages between the two groups (152 eyes; RR 0.62, 95% CI 0.24 to 1.56; RD -0.05, 95% CI -0.16 to 0.05; very low quality evidence). The risk of delayed systemic adverse effects with either of the drugs is, however, not known.

Authors' conclusions

Implications for practice: Intravitreal bevacizumab reduces the risk of refractive errors during childhood when used as monotherapy while intravitreal pegaptanib reduces the risk of retinal detachment when used in conjunction with laser therapy in infants with type 1 ROP. Quality of evidence was, however, low for both the outcomes because of the risk of detection and other biases. Effect on other critical outcomes and, more importantly, the long-term systemic adverse effects of the drugs are not known. The insufficient data precludes strong conclusions favouring routine use of intravitreal anti-VEGF agents in preterm infants with type 1 ROP.

Implications for research: Further studies are needed to evaluate the effect of anti-VEGF agents on structural and functional outcomes in childhood and delayed systemic adverse effects such as myocardial dysfunction and adverse neurodevelopmental outcomes.

PLAIN LANGUAGE SUMMARY

Anti-vascular endothelial growth factor (VEGF) drugs for treatment of retinopathy of prematurity

Background

Retinopathy of prematurity (ROP) is a vascular disorder of the immature retina that can result in impairment of vision and even blindness in premature infants. It is treated primarily by removal of the part of the retina without any blood vessels ('avascular' retina) by cryotherapy or laser therapy. Though these treatments result in a significant improvement in long-term outcomes, the results are far from perfect. In addition, they cause permanent loss of the peripheral visual field. Recently, studies have been done to evaluate the use of anti-VEGF agents to treat ROP. These agents inhibit the action of vascular endothelial growth factor (VEGF), a key regulator of new vessel formation in fetal life. Animal studies had shown significant reduction in the neovascular response following injection of anti-VEGF antibodies into the vitreous cavity of the eyes ('intravitreal' therapy).

Study characteristics

We searched scientific databases in January 2016 for studies evaluating the efficacy and safety of intravitreal therapy with anti-VEGF agents in preterm infants with ROP. We identified three randomized controlled trials involving 239 infants. Two trials compared intravitreal bevacizumab with conventional laser therapy. The third trial compared intravitreal pegaptanib *plus* laser therapy with laser/cryotherapy alone.

Key results

The results suggest that intravitreal bevacizumab reduces the risk of refractive errors (high myopia) during childhood while intravitreal pegaptanib used in conjunction with laser therapy reduces the risk of retinal detachment. Effects on other critical outcomes including delayed side effects like stroke are not known. Further studies are needed to assess these outcomes.

Quality of the evidence

The quality of the evidence was graded as very low or low for most of the key outcomes.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

Intravitreal anti-VEGF therapy compared to conventional laser/cryotherapy in preterm infants with type 1 ROP

Patient or population: preterm infants with type 1 ROP
Settings:
Intervention: intravitreal anti-VEGF therapy
Comparison: conventional laser/cryotherapy

Outcomes*	Illustrative comparative risks# (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	**Corresponding risk**			
	conventional laser/ cryotherapy	**intravitreal anti-VEGF therapy**			
Structural outcome - retinal detachment	Study population		RR 1.04 (0.21 to 5.13)	143 (1 RCT)	⊕◯◯◯ VERY LOW [1,2,3]
	27 per 1000	28 per 1000 (6 to 141)			
Refractive error - very high myopia - at 30 months of age (unit of analysis: eyes)	Study population		RR 0.06 (0.02 to 0.2)	211 (1 RCT)	⊕⊕◯◯ LOW [1,4]
	416 per 1000	25 per 1000 (8 to 83)			
Mortality before discharge from primary hospital	Study population		RR 1.5 (0.26 to 8.75)	150 (1 RCT)	⊕⊕◯◯ LOW [2,3,5]
	27 per 1000	40 per 1000 (7 to 233)			
Mortality at 30 months of age	Study population		RR 0.86 (0.3 to 2.45)	150 (1 RCT)	⊕⊕◯◯ LOW [2,3,5]
	93 per 1000	80 per 1000 (28 to 229)			

			RR 0.34	286	⊕◯◯◯
Local adverse effects - corneal opacity requiring corneal transplant (unit of analysis: eyes)	Study population		(0.01 to 8.26)	(1 RCT)	VERY LOW [1,2,3,4]
	7 per 1000	2 per 1000 (0 to 57)			
Local adverse effects - lens opacity requiring cataract removal (unit of analysis: eyes)	Study population		RR 0.15 (0.01 to 2.79)	286 (1 RCT)	⊕◯◯◯ VERY LOW [1,2,3,4]
	21 per 1000	3 per 1000 (0 to 57)			
Recurrence of ROP by 54 weeks PMA	Study population		RR 0.22 (0.08 to 0.62)	143 (1 RCT)	⊕⊕⊕◯ MODERATE [1]
	260 per 1000	57 per 1000 (21 to 161)			

*Only the outcomes for which data are available are reported here;

#The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

[1]Outcome assessment not blinded

[2]95% CI around the pooled estimate includes both 1) no effect and 2) appreciable benefit or appreciable harm

[3]Number of events too small

[4]Serious risk of bias in analysis (unit of analysis error)

[5]Outcome assessment not blinded but outcome is objective

BACKGROUND

Description of the condition

Retinopathy of prematurity (ROP) is one of the major avoidable causes of childhood blindness in both developed and developing countries (Gilbert 2008). Essentially a neovascularising disease of the retina, ROP occurs almost exclusively in preterm very low birth weight (VLBW) infants. With improved survival of the VLBW infants, the absolute number of children with visual impairment secondary to ROP has increased in recent years (Gilbert 2005; Mantagos 2009).

The incidence of ROP varies inversely with gestation and weight at birth. A multicenter study of infants born between 1986 and 1987 reported that 81.6% of infants weighing less than 1000 grams (g) developed ROP while only 46.9% of those weighing 1000 g to 1250 g had ROP (Palmer 1997).

The other risk factors of ROP include exposure to varying oxygen concentrations, hypercapnia, anaemia, acidosis, chronic lung disease and intraventricular haemorrhage (IVH) (Ashton 1953; Smith 2003; Tasman 2006).

The predisposition of preterm infants to development of ROP relates to their immature retinal vasculature. In humans, retinal vascularisation begins at about 12 weeks and is completed by 36 weeks to 40 weeks of gestation. Normally, the blood vessels develop from the optic disc and then progress outwards towards the ora serrata. Infants born before this period will, therefore, have an immature retina with a peripheral avascular zone. ROP develops if there is a disruption in the new vessel formation - angiogenesis - in this zone. The disruption of angiogenesis has been found to occur in two sequential phases: a vaso-obliterative phase followed by a vaso-proliferative phase (Ashton 1954). In the vaso-obliterative phase (phase 1), the normally high arterial oxygen saturation in the postnatal life coupled with hyperoxia secondary to oxygen supplementation leads to involution and loss of formed blood vessels. In the second phase (phase 2), the relatively hypoxic environment due to ischaemia caused by vessel loss coupled with the high metabolic demands of the avascular retina leads to up regulation of various angiogenic factors resulting in abnormal neovascularisation. In most infants, the new vessels formed regress without leaving any sequelae. However, in some infants the neovascularisation goes unchecked leading to retinal scarring, traction and finally detachment.

The extent and severity of ROP are traditionally described in terms of location (zones; I to III), severity (stages; 1 to 5), extent (clock hours; 1 to 12), and vascular dilatation and tortuosity (plus disease) according to the International Classification of ROP definitions (Committee for Classification of ROP 1984). In addition to defining the progression of the disease, this classification also serves as a guide for surgical intervention. In 2005, the classification was revised to include aggressive posterior ROP (AP-ROP), pre-plus disease, and a practical way to estimate the extent of zone I with the indirect ophthalmoscope. Concomitantly, the recommendations for treatment were also revised based on the results of the Early Treatment for Retinopathy of Prematurity trial (ET-ROP Group 2003). The new recommendations place more emphasis on the presence of plus disease, rather than the number of clock hours, to decide upon the need for treatment (American Academy of Pediatrics 2006). Accordingly, treatment is initiated for the following retinal ndings (type 1 ROP):

1. Zone I ROP: any stage with plus disease
2. Zone I ROP: stage 3 - no plus disease
3. Zone II ROP: stage 2 or stage 3 with plus disease

The current treatment strategy for infants with type 1 ROP involves peripheral retinal ablation by either cryotherapy or laser therapy. Both techniques result in significant improvement in the structural and functional outcomes - a Cochrane review reported significant reduction in the risk of early unfavourable retinal structure from 47.9% to 28.1% and unfavourable visual acuity in early childhood from 63% to 50.6% following peripheral retinal ablation (Andersen 1999). However, in a small but significant proportion of preterm infants, the disease progresses despite treatment. Also, visual fields are slightly smaller in eyes subjected to peripheral retinal ablation as compared to 'control' eyes (Andersen 1999). Moreover, the ablation techniques are cumbersome and require sedation, general anaesthesia, or both. This has led to the quest for simpler and more effective treatment strategies.

Description of the intervention

Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis in fetal life. In the normally developing retina, VEGF is released in response to the higher oxygen demand of the retinal tissue which leads to the development of blood vessels from the optic nerve to the periphery. In preterm infants with disrupted angiogenesis, however, the expression and levels of VEGF differ markedly in the two different phases. While the levels are suppressed in the vaso-obliterative phase, there is an over production/ expression of VEGF leading to abnormal vascular proliferation in the vaso-proliferative phase. Hugely elevated levels of VEGF have been documented in the vitreous cavity of eyes with stage 4 ROP (Lashkari 2000).

The key role played by VEGF in inducing retinal neovascularisation prompted researchers to explore the role of anti-VEGF drugs in the management of ROP. Intravitreal injection of neutralizing anti-VEGF antibodies had demonstrated significant reduction in the neovascular response in animal studies (Aiello 1995). Two anti-VEGF drugs namely, pegaptanib sodium and ranibizumab, had been approved by the United States Food and Drug Administration (FDA) for intraocular use in adults in neovascular and age-related macular degeneration (AMD). The third inhibitor - bevacizumab - is being used off-label for intraocular injection in adults with similar results. Unfortunately, none of these drugs have been approved for intraocular use in children to date (Mantagos

2009). However, given the limitations of existing treatment strategies (see above), many investigators have evaluated the off-label use of these agents in infants with ROP (Shah 2007; Kong 2008; Mintz-Hittner 2008). These studies - predominantly case-series and retrospective studies - used one or more of the following approaches to evaluate the efficacy of VEGF inhibitors:

1. monotherapy - using an anti-VEGF drug instead of cryo/laser therapy;

2. combination therapy - using anti-VEGF simultaneously with cryo/laser therapy;

3. rescue therapy - using anti-VEGF in infants with progression of the disease despite adequate treatment and in the rare instances where the infant presents with advanced ROP (stage 4 or more).

While the efficacy of anti-VEGF drugs in ROP was demonstrated in most of the studies, their safety is yet to be established. Though no significant adverse events have been reported so far (except for tractional retinal attachment in an occasional infant with advanced ROP), concerns still remain regarding their potential local and systemic adverse effects. By inhibiting VEGF, a key factor in regulation of angiogenesis in the developing retina as well as the central nervous system, these drugs could result in significant local and systemic adverse effects. Indeed, there have been concerns regarding the risk of cerebrovascular accidents following intravitreal ranibizumab injections in adults with AMD (Ueta 2009). Though bevacizumab, the most frequently tested drug in ROP, has a lower risk of systemic absorption following intravitreal injections, the distinct possibility of its systemic effects cannot be ruled out in preterm infants with immature, and often impaired, blood-retinal barrier (Law 2010).

The risk of systemic absorption, though small, brings its own complexity in the methods of randomisation and analysis in trials involving a locally administered drug. For a drug with truly local action, one can randomise either the study subject or a local body part of the subject to intervention and control groups. In the former, infants would be randomized - both eyes of the infants (if needed) would receive the intervention or control therapy as per the group allocation. In the latter, eyes of the infants would be randomized - one eye would receive the intervention while the other eye would receive 'control' therapy. However, if the drug is likely to have systemic effects (like most anti-VEGF agents), randomising the body part is not an ideal method of randomisation.

Why it is important to do this review

Treatment of ROP, to date, is largely surgical - by cryotherapy or by laser therapy. Although these treatments result in a significant improvement in long-term visual outcomes, the results are far from perfect. Despite appropriate treatment, progression to tractional retinal detachment occurs in 10% to 15% of infants with threshold disease (ET-ROP Group 2003). In addition, the ablation procedures invariably cause permanent loss of the peripheral visual field. Simple, effective, and less destructive treatment strategies would definitely be preferable to these procedures.

The recent reports of success following bevacizumab use have stimulated various investigators to conduct clinical studies on the efficacy of this intervention. However, many of these studies are not powered to detect any serious adverse events - local or systemic - in the enrolled infants. Also, most have not systematically documented the risk of recurrence of ROP at a later age or examined the duration of follow-up required to detect recurrence following intravitreal therapy. Given the protracted course of the disease and the short half-life of anti-VEGF drugs, the potential risk of recurrence is high (Wong 2015).

An earlier systematic review on the use of bevacizumab for severe ROP that included only case reports/case-series and retrospective studies found considerable variability in how bevacizumab is used for the treatment of ROP (Micieli 2009). It concluded that 'further randomized control trials are warranted'. The purpose of this review is to identify all the available randomized controlled trials (RCT) on intravitreal anti-VEGF therapy and to systematically analyse their results.

O B J E C T I V E S

To evaluate the efficacy and safety of anti-VEGF drugs when used either as monotherapy, i.e. without concomitant cryotherapy or laser therapy or in combination with planned cryo/laser therapy in preterm infants with type 1 ROP (defined as zone I any stage with plus disease, zone I stage 3 with or without plus disease or zone II stage 2 or 3 with plus disease).

M E T H O D S

Criteria for considering studies for this review

Types of studies

Randomised or quasi-randomised controlled trials that evaluated the efficacy or safety of administration, or both, of anti-VEGF agents in human preterm infants were considered for inclusion in this review. We included only those trials that used VEGF inhibitors either alone (i.e. monotherapy) or in combination with cryo/laser therapy (i.e. combination therapy). We excluded those studies that used these drugs when other treatments such as cryo/laser therapy or vitrectomy had failed ('rescue therapy').

Types of participants

Studies that enrolled preterm (< 37 weeks' gestation at birth) infants with type 1 ROP at enrolment were considered for inclusion. Type 1 ROP was defined as zone I any stage with plus disease, zone I stage 3 ROP with or without plus or zone II stage 2 or 3 ROP with plus disease (ET-ROP Group 2003). For the purpose of this review, we excluded those studies that enrolled infants with more advanced ROP - stage 4 or more - at the time of enrolment (irrespective of the treatment strategy employed).

Types of interventions

Objective 1

Intervention: Administration of any anti-VEGF agent by intravitreal route.
Control: Cryotherapy/laser therapy.

Objective 2

Intervention: Intravitreal administration of VEGF inhibitors within seven days (before or after) of a planned laser or cryotherapy.
Control: Cryotherapy/laser therapy alone.

Types of outcome measures

Primary outcomes

1. Functional outcome - blindness or severe visual impairment (acuity ≤ 20/200) at 6 months to 12 months of corrected age.
2. Structural outcome - progression to retinal detachment involving the macula.

Secondary outcomes

1. Functional outcome(s) at 6 months to 12 months of corrected age:
    i) amblyopia;
    ii) nystagmus and/or;
    iii) refractive error in either eye.
2. Unfavourable structural outcomes, assessed at 6 months to 12 months of corrected age, and defined as:
    i) retinal fold involving the macula or;
    ii) retinal detachment involving zone 1 of the posterior pole and/or;
    iii) retrolental tissue or 'mass' obscuring the view of the posterior pole.
3. Childhood unfavourable visual acuity, assessed at four years to six years, and defined as absence of vision or Snellen visual acuity of 20/200 or worse.
4. Mortality measured as:

    i) death before discharge from the primary hospital;
    ii) death before two years corrected age.
5. Adverse neurodevelopmental outcomes at 18 months to 24 months of corrected age:
    i) cerebral palsy and/or;
    ii) moderate to severe developmental delay as assessed on performance in formal neurodevelopmental testing such as Bayley scale.
6. Local adverse effects such as conjunctival haemorrhage, vitreous haemorrhage, and endophthalmitis after the procedure.
7. Acute systemic effects such as apnoea requiring respiratory support and cardiorespiratory arrest during or immediately after the treatment procedure.
8. Delayed systemic effects such as cerebrovascular accidents (stroke) and myocardial dysfunction (based on echocardiographic parameters such as ejection fraction and fractional shortening) diagnosed in the first 24 months of life.
9. Parental satisfaction regarding the treatment procedure employed (in Likert or other such scales).
10. Recurrence of ROP requiring re-treatment by 54 weeks to 55 weeks PMA.

Search methods for identification of studies

Electronic searches

We used the criteria and standard methods of the Cochrane Collaboration and the Cochrane Neonatal Review Group (see the Cochrane Neonatal Group search strategy for specialized register). We conducted a comprehensive search including: Cochrane Central Register of Controlled Trials (CENTRAL) in *The Cochrane Library* (Issue 1, 2016), MEDLINE (1966 to January 1, 2016) via PubMed, EMBASE (1980 to January 1, 2016) and CINAHL (1982 to January 1, 2016) using the following search terms: (bevacizumab OR Avastin OR ranibizumab OR pegaptanib sodium OR anti-angio* OR angiogenesis inhibitors), plus database-specific limiters for RCTs and neonates (see Appendix 1 for the full search strategies for each database). We limited the searches to human studies. We did not apply any language restrictions. We searched clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; the World Health Organization's International Trials Registry and Platform www.whoint/ictrp/search/en/, and the ISRCTN Registry).

Searching other resources

We searched for unpublished studies by handsearching the conference proceedings of the Society for Pediatric Research (2002-2013) and American Academy of Ophthalmology Annual Meeting (1999-2013). We also searched the reference lists of all studies identified by the above methods.

Data collection and analysis

Selection of studies

Three review authors (MJS, JS and MM) independently searched and identified the eligible trials based on the following characteristics: study population (preterm infants with type 1 ROP), study intervention (administration of anti-VEGF drugs with or without laser/cryotherapy), and study design (RCTs).

The authors screened the titles and abstracts to identify potentially relevant citations. The full text of the article was retrieved and reviewed if relevance could not be ascertained by screening the title and the abstract. The review authors independently assessed the eligibility of the studies by filling out eligibility forms designed in accordance with the specified inclusion criteria. We resolved any disagreement by discussion.

We contacted the study investigators for additional information or clarification of the method of randomisation, patient characteristics, details of interventions, definitions of events, additional relevant outcomes and losses to follow-up, as necessary.

Data extraction and management

Data extraction was done using a data extraction form that was designed and pilot tested by the review authors. We extracted information regarding:

1. study setting (for example, country and settings);
2. study intervention;
3. sample size;
4. length of follow-up;
5. randomisation procedure;
6. risk of different biases (see Assessment of risk of bias in included studies below);
7. outcomes as listed above.

For dichotomous outcomes, we extracted the total number of participants for each group and the number of participants experiencing an event. For continuous outcomes, we extracted mean, standard deviation (or data required to calculate this) and the total number of participants for each group.

Assessment of risk of bias in included studies

Three review authors (MJS, JS and MM) independently assessed the methodological quality of all the included studies using The Cochrane 'Risk of bias' tool (Higgins 2011; Higgins 2011a). The items in the tool included the following:

1. sequence generation (allocation sequence adequately generated);
2. allocation sequence concealment (allocation adequately concealed);
3. blinding (knowledge of the allocated intervention adequately prevented during the study);

4. incomplete outcome data (incomplete outcome data adequately addressed);
5. selective outcome reporting (reports of the study free of suggestion of selective outcome reporting);
6. other potential sources of bias (study apparently free of other problems that could put it at a high risk of bias).

A judgement of 'Yes' indicates low risk of bias, 'No' indicates high risk of bias, and 'Unclear' indicates unclear or unknown risk of bias. We contacted the study authors when insufficient information was provided to determine the risk of bias. Risk of bias was summarised for the primary outcomes.

In addition to assessing the risk of bias, we also applied the GRADE system to assess the quality of the body of the evidence (Schünemann 2011). According to GRADE, the domains that may decrease the quality of the evidence include:

1. study design;
2. risk of bias;
3. inconsistency of results;
4. indirectness (non-generalisability);
5. imprecision (insufficient data);
6. other factors (e.g. reporting bias).

The quality of the evidence was reduced by one level for each of the domains that was encountered. The following levels of evidence were defined.

- High quality evidence: Further research is very unlikely to change our confidence in the estimate of effect. There are consistent findings among 75% of RCTs with low risk of bias that are generalisable to the population in question. There are sufficient data, with narrow confidence intervals. There are no known or suspected reporting biases (all of the domains are met).

- Moderate quality evidence: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate (one of the domains is not met).

- Low quality evidence: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate (two of the domains are not met).

- Very low quality evidence: We are very uncertain about the estimate (three of the domains are not met).

We used the GRADE profiler (GRADEpro GDT) to import data from Review Manager 5.3 (RevMan) (RevMan 2014) to create the 'Summary of findings' tables. These tables provide outcome-specific information concerning the overall quality of evidence from studies included in the comparison, the magnitude of effect of the interventions examined, and the sum of available data on the outcomes we considered.

Measures of treatment effect

We used the standard methods of the Neonatal Review Group to synthesise the data. We expressed effects as risk ratio (RR), risk difference (RD) and 95% confidence intervals (CI) for categorical

data, and mean difference (MD) and 95% CI for continuous data. For significant differences, we also calculated the number needed to treat for an additional beneficial outcome (NNTB) based on 1/RD. We used the fixed-effect model for pooling the results of individual studies.

Unit of analysis issues

We anticipated that the units of randomisation and analysis in the included trials would be individual infants and not eyes (it is difficult to randomise eyes because intravitreal anti-VEGF can be absorbed into the systemic circulation). However, if a given study had randomized eyes and not infants, we intended to use the study data but refrained from pooling it with data of studies that had randomized infants. We *a priori* decided to use the eye-level data (and not infant-level data) in these studies, i.e. incidence of outcomes in eyes randomized to anti-VEGF vs. incidence of outcomes in eyes randomized to the control group; consequently, individual-level outcomes such as mortality were not considered in these studies. We *a priori* assumed that the beneficial effect, if any, would be diluted in them, i.e. the effect size would be closer to the null effect, if systemic absorption of anti-VEGF agents were to occur (because the eye randomized to control group would be exposed to both anti-VEGF agents and 'control' treatment).

If a given study had randomized infants but provided the outcome data for eyes, we planned to contact the authors to obtain infant-level data so as to avoid unit of analysis error - using eyes as the denominator without adjusting for non-independence between the eyes may result in spuriously precise results, i.e. narrow confidence intervals similar to that seen in cluster randomized trials when the clusters are randomized but the outcomes are analysed at the individual level without adjusting for 'cluster' effect (Higgins 2011b). If that information could not be obtained, we used the data for eyes but mentioned up-front that the analysis referred to eyes and not infants.

Dealing with missing data

At the outcome level, if the data were measured but not reported, we planned to request such data from study authors. If there was a discrepancy in the number randomized and the number analysed in each treatment group, we calculated and reported the percentage lost to follow-up in each group.

Assessment of heterogeneity

We intended to examine for heterogeneity between trial results by inspecting the forest plots and quantifying the impact of heterogeneity in any meta-analysis using a measure of the degree of inconsistency in the studies' results (Deeks 2011).

We estimated the proportion of total statistical heterogeneity not explained by chance using the I^2 statistic (Higgins 2003). I^2 (cal-culated as $I^2 = 100\% \times (Q - df)/Q$; where Q is Cochrane's heterogeneity statistic and df is the degrees of freedom) lies between 0% and 100%.

Data synthesis

Quantitative data was entered into RevMan (RevMan 2014) and analysed using the standard methods of Cochrane and Cochrane Neonatal. We used the Mantel-Haenszel method for estimates of typical RR and RD. We analysed continuous outcomes using the inverse variance method.

Subgroup analysis and investigation of heterogeneity

Considerations of clinical diversity included assessment of differences in nature of the surgical intervention, type and extent of disease and the number and route of administration of VEGF inhibitors. Accordingly, we planned to analyse the studies based on differences in the following pre-planned subgroups.
- Nature of retinal ablation procedure (cryotherapy versus laser therapy)
- Location of ROP at enrolment (zone I versus zone II)
- Severity of ROP at enrolment (AP-ROP versus others)
- Specific anti-VEGF agent administered
- Number of doses of anti-VEGF drug (single versus multiple)
- Birth weights of the enrolled infants (< 1250 g versus $\geq$ 1250 g)

Sensitivity analysis

We intended to conduct a sensitivity analysis to investigate the robustness of the results for the primary outcome by excluding trials at high risk of bias or with drop-out rates of more than 10% (overall).

R E S U L T S

Description of studies

See:Characteristics of included studies

Results of the search

A total of 1518 records were retrieved from the literature, of which 1513 were excluded after scanning the title or abstract, or both (Figure 1). Three randomized trials fulfilled the eligibility criteria (BEAT-ROP Trial 2011; Autrata 2012; Lepore 2014) and were included in the review. Two articles remain in studies awaiting classification. No article was excluded after full-text assessment.

Figure 1. Study flow diagram.
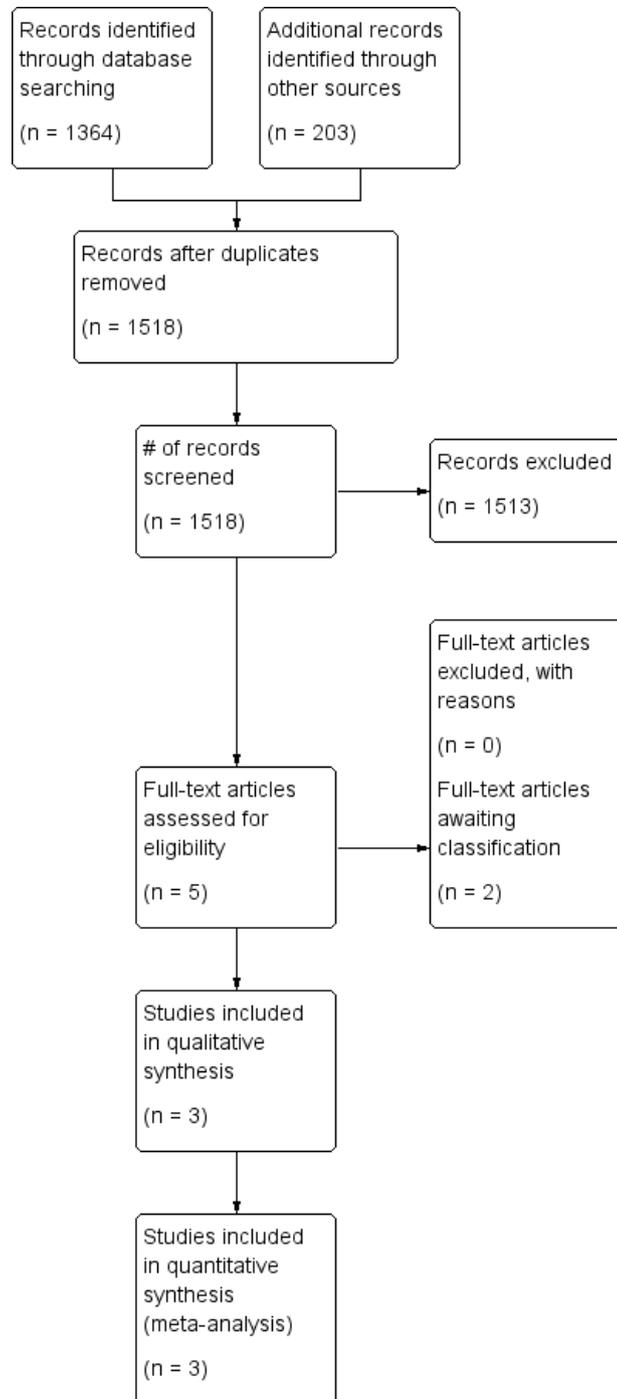
Included studies

Of the three included studies, two compared intravitreal bevacizumab monotherapy with conventional laser therapy (BEAT-ROP Trial 2011; Lepore 2014) while the third compared intravitreal pegaptanib *plus* conventional laser therapy with laser and cryotherapy (Autrata 2012). Two trials (BEAT-ROP Trial 2011; Autrata 2012) randomized infants whereas the third randomized eyes of the infants to the two groups (Lepore 2014).

BEAT-ROP Trial 2011 was a multi-centre randomized trial conducted at 15 hospitals in the United States of America (BEAT-ROP Trial 2011). It enrolled 150 preterm infants with zone I or zone II posterior stage 3+ ROP and randomly assigned them to receive intravitreal bevacizumab (0.625 mg in 0.025 ml of solution) or conventional laser therapy, bilaterally. The primary outcome was treatment failure, defined as the recurrence of neovascularisation in one or both eyes requiring re-treatment, by 54 weeks' postmenstrual age (PMA). RetCam (retinal imaging) photographs taken at different time points were evaluated to document recurrence of ROP. The study investigators reported the refractive outcomes at 30 months of age in a separate publication in 2014 by Geloneck (see BEAT-ROP Trial 2011). Of the originally enrolled 150 infants, 131 infants underwent cycloplegic retinoscopic refraction to assess refractive outcomes at this age.

Autrata 2012 enrolled 76 preterm infants with zone I or zone II posterior stage 3+ ROP, admitted in a University Hospital in the Czech Republic. Enrolled infants were randomly assigned to receive intravitreal pegaptanib (0.3 mg in 0.02 ml of solution) *plus* conventional diode laser therapy or laser therapy combined with cryotherapy, bilaterally. The primary outcome was treatment success defined as absence of recurrence of stage 3+ ROP in one or both eyes by 55 weeks' PMA. RetCam photographs taken at different time points were evaluated to document recurrence of ROP. Infants who were randomized to the intervention group and had recurrence were given an additional intravitreal pegaptanib injection; those in the laser-plus-cryotherapy group did not receive pegaptanib for recurrence.

Lepore 2014 conducted a single-centre randomized trial and enrolled 13 infants with type 1 ROP in zone I in both eyes who required treatment according to ET-ROP criteria (ET-ROP Group 2003). One eye of the enrolled infants was randomized to receive an intravitreal injection of 0.5 mg bevacizumab while the fellow eye received conventional laser photoablation. The eye assigned to conventional laser peripheral ablation was treated first. The primary outcome was presence of retinal and choroidal abnormalities on fluorescein angiography (FA) at nine months of age. After treatment, binocular indirect ophthalmoscopy and RetCam imaging were performed every three days, and FA was performed every two weeks until discharge. FA was done again at nine months of age under general anaesthesia.

Further details of the three studies are provided in the Characteristics of included studies table.

Excluded studies

No study was excluded following full-text assessment.
The studies of Kong 2015 and Moran 2014 are awaiting further assessment.

Risk of bias in included studies

Quality assessments are detailed in Characteristics of included studies table and are also summarised in Figure 2.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Allocation

BEAT-ROP Trial 2011 reported an adequate allocation concealment method whereas the other two trials (Autrata 2012; Lepore 2014) did not report details of allocation concealment.

Blinding

Given the nature of the intervention, blinding of the intervention was not possible in any of the studies.

BEAT-ROP Trial 2011 constituted a panel of six independent experts to examine the photographs taken at 54 weeks' PMA, and masked them to the treatment assignments by cropping the photographs to include only the optic disk and macula without laser marks. This enabled the study investigators to perform blinded assessment of some of the secondary outcomes (e.g. macular dragging) but not the primary outcome of the study. Paediatric ophthalmologists who performed the cycloplegic retinoscopic refractions to assess the refractive errors at 30 months of age were not masked to the treatment assignments.

It is not clear if the outcome assessors were masked to the group allocation in the other two trials (Autrata 2012; Lepore 2014).

Incomplete outcome data

Two trials reported no loss to follow-up until 54 weeks' PMA (BEAT-ROP Trial 2011; Autrata 2012). About 17% of eligible infants were lost to follow-up at 30 months of age in BEAT-ROP Trial 2011. There was no loss to follow-up at nine months of age in Lepore 2014.

Selective reporting

BEAT-ROP Trial 2011 reported all the outcomes listed in the protocol (see NCT00622726 in BEAT-ROP Trial 2011). The other two trials had unclear risk of reporting bias. We could not refer to the study protocol in Autrata 2012; secondary outcomes were not provided in the study protocol of Lepore 2014.

Other potential sources of bias

In Lepore 2014, eyes of enrolled infants were randomized. If there was significant systemic absorption of bevacizumab, the eye randomized to control group would have been exposed to both anti-VEGF agents and control treatment resulting in better outcomes in that eye.

We intended to assess the likelihood of potential publication bias using funnel plots, provided there were at least 8 to10 trials (Sterne 2011).

Effects of interventions

See: **Summary of findings for the main comparison** Intravitreal anti-VEGF therapy compared to conventional laser/cryotherapy in preterm infants with type 1 ROP; **Summary of findings 2** Anti-VEGF combined with laser/cryotherapy compared to laser/cryotherapy in preterm infants with type 1 ROP

Comparison 1: Anti-VEGF vs cryo/laser therapy ('monotherapy')

Two trials (BEAT-ROP Trial 2011; Lepore 2014) compared intravitreal bevacizumab with conventional laser therapy. While BEAT-ROP Trial 2011 randomized preterm infants with stage 3+ ROP in zone I or zone II posterior, Lepore 2014 randomized eyes of infants with type 1 ROP in zone I. Because the unit of randomisation was different in the two studies (infants versus eyes), we did not attempt to pool their results.

Primary outcomes

Functional outcome - blindness or severe visual impairment at 6 to 12 months of corrected age

Neither of the studies reported this outcome.

Structural outcome - progression to retinal detachment involving the macula (Outcome 1.1)

BEAT-ROP Trial 2011 did not report any difference in the incidence of complete or partial retinal detachment between the two groups (RR 1.04, 95% CI 0.21 to 5.13; RD 0.00, 95% CI -0.06 to 0.07; Analysis 1.1). Only a small number of infants - two infants each in the two groups - had retinal detachment. The quality of evidence was graded very low because of the small number of events and the risk of detection bias (Summary of findings for the main comparison). No separate data was available for the risk of retinal detachment involving only the macula.

In the other trial (Lepore 2014), one eye (8.5%) treated with conventional laser progressed to complete retinal detachment four weeks after treatment while none of the eyes randomized to intravitreal bevacizumab had complete detachment (RR 0.33, 95% CI 0.01 to 7.50; RD -0.08, 95% CI -0.27 to 0.11; Analysis 1.2).

Secondary outcomes

Functional outcome(s) - refractive error at 30 months of age (Outcomes 1.2 to 1.3)

One study (BEAT-ROP Trial 2011) reported the outcome using eyes as the denominator. The risk of very high myopia, defined as -8 diopters (D) or more, was significantly lower in the eyes of infants randomized to intravitreal bevacizumab (RR 0.06, 95% CI 0.02 to 0.20; RD -0.40, 95% CI -0.50 to -0.30)(Analysis 1.3).

The magnitude of benefit was almost the same in both zone I and zone II posterior ROP. The quality of evidence was graded low because of the unit of analysis error and the risk of detection bias (Summary of findings for the main comparison).

The mean spherical equivalent refractive error was also significantly less in eyes of infants who received intravitreal bevacizumab (mean difference [MD] 5.68 Diopter, 95% CI 4.33 to 7.02). The magnitude of difference was almost the same in both zone I (MD 6.93 Diopter, 95% CI 4.26 to 9.60) and zone II posterior ROP (MD 5.25 Diopter, 95% CI 3.69 to 6.81)(Analysis 1.4).

Unfavourable structural outcomes, assessed at 6 to 12 months of corrected age

Neither of the studies reported this outcome.

Childhood unfavourable visual acuity, assessed at four to six years

Neither of the studies reported this outcome.

Mortality before discharge from the primary hospital and at 30 months of age (Outcomes 1.4 to 1.5)

BEAT-ROP Trial 2011 reported no difference in the risk of mortality between the two groups - either before discharge from the primary hospital (RR, 1.50; 95% CI 0.26 to 8.75)(Analysis 1.5) or at a mean age of 30 months (RR 0.86, 95% CI 0.30 to 2.45)(Analysis 1.6). The number of events was, however, very small.

Adverse neurodevelopmental outcomes at 18 to 24 months of corrected age

Neither of the studies reported this outcome.

Local adverse effects (Outcomes 1.6 to 1.7)

One study (BEAT-ROP Trial 2011) reported the outcome using eyes as the denominator. There was no significant difference in the incidence of corneal opacity requiring corneal transplant (RR 0.34, 95% CI 0.01 to 8.26) (Analysis 1.7) or lens opacity requiring cataract removal (RR 0.15, 95% CI 0.01 to 2.79) (Analysis 1.8) between the two groups. The study did not report the risk of conjunctival haemorrhage, vitreous haemorrhage, or endophthalmitis in the two groups.

Acute systemic effects during or immediately after the treatment procedure

Neither of the studies reported this outcome.

Delayed systemic effects

Neither of the studies reported this outcome.

Parental satisfaction regarding the treatment (in Likert or other such scales)

Neither of the studies reported this outcome.

Additional outcomes

Recurrence of ROP by 54 weeks' postmenstrual age

One study (BEAT-ROP Trial 2011) reported this outcome. Infants randomized to intravitreal bevacizumab therapy had significantly lower risk of recurrence of ROP requiring retreatment by 54 weeks' PMA when compared to infants who received conventional laser therapy (RR 0.22, 95% CI 0.08 to 0.62; RD -0.20, 95% CI -0.31

to -0.09; NNTB 5; 95% CI 3 to 11) (Analysis 1.9). The quality of evidence for this outcome was graded moderate (Summary of findings for the main comparison). On subgroup analysis, the difference was found to be significant only in infants with Zone I ROP.

Comparison 2: Anti-VEGF plus cryo/laser therapy ('combination therapy') vs cryo/laser therapy alone

Autrata 2012 compared intravitreal pegaptanib *plus* conventional laser therapy with laser and cryotherapy in preterm infants with stage 3+ ROP.

Primary outcomes

Functional outcome - blindness or severe visual impairment at 6 to 12 months of corrected age

The study did not report this outcome.

Structural outcome - progression to retinal detachment involving the macula (Outcome 2.1)

The study reported the outcome using eyes as the denominator. The risk of complete or partial retinal detachment was significantly lower in the eyes of infants randomized to intravitreal pegaptanib *plus* laser therapy (RR 0.26, 95% CI 0.12 to 0.55; RD -0.29, 95% CI -0.42 to -0.16; one study, 152 infants) (Analysis 2.1). The quality of evidence was graded low because of the unit of analysis error and the risk of detection bias (Summary of findings 2).

No separate data was available for the risk of retinal detachment involving only the macula in the two groups.

Secondary outcomes

Functional outcome(s) - refractive error at 6 to 12 months of age or later

The study did not report this outcome.

Unfavourable structural outcomes at 6 to 12 months of age

The study did not report this outcome.

Childhood unfavourable visual acuity

The study did not report this outcome.

Mortality before discharge from the primary hospital and at 30 months of age

The study did not report this outcome.

Adverse neurodevelopmental outcomes at 18 to 24 months of corrected age

The study did not report this outcome.

Local adverse effects (Outcomes 2.2)

The study reported the outcome using eyes as the denominator. There was no significant difference in the risk of perioperative retinal haemorrhages after laser therapy between the two groups (RR 0.62, 95% CI 0.24 to 1.56; one study, 152 infants) (Analysis 2.2). The study did not report the risk of conjunctival haemorrhage or vitreous haemorrhage in the two groups.

The study reported that "no systemic or significant ocular complications of intravitreal pegaptanib injections, such as endophthalmitis or RD were found during the follow-up period after treatment" but did not provide the corresponding data for the other group.

Acute systemic effects during or immediately after the treatment procedure

The study did not report this outcome (see 'Local adverse effects' above).

Delayed systemic effects

The study did not report this outcome.

Parental satisfaction regarding the treatment (in Likert or other such scales)

The study did not report this outcome.

Additional outcomes

Recurrence of ROP by 55 weeks' postmenstrual age (Outcome 2.3)

Infants randomized to intravitreal pegaptanib *plus* laser therapy had significantly lower risk of recurrence of ROP by 55 weeks' PMA when compared to those randomized to laser therapy with cryotherapy (RR 0.29, 95% CI 0.12 to 0.70; RD -0.35, 95% CI -0.55 to -0.16; NNTB 3; 95% CI 2 to 6; one study, 76 infants) (Analysis 2.3). The quality of evidence for this outcome was graded low because of the risk of detection bias and the unclear risk of selection bias (Summary of findings 2).

ADDITIONAL SUMMARY OF FINDINGS [Explanation]

Anti-VEGF combined with laser/cryotherapy compared to laser/cryotherapy in preterm infants with type 1 ROP

Patient or population: preterm infants with type 1 ROP
Settings:
Intervention: anti-VEGF combined with laser/cryotherapy
Comparison: laser/cryotherapy

Outcomes*	Illustrative comparative risks# (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	**Corresponding risk**			
	laser/cryotherapy	**anti-VEGF combined with laser/cryotherapy**			
Structural outcome - retinal detachment (unit of analysis: eyes)	Study population		RR 0.26 (0.12 to 0.55)	152 (1 RCT)	⊕⊕◯◯ LOW [1,2,3]
	393 per 1000	102 per 1000 (47 to 216)			
Local adverse effects - perioperative retinal haemorrhages (unit of analysis: eyes)	Study population		RR 0.62 (0.24 to 1.56)	152 (1 RCT)	⊕◯◯◯ VERY LOW [1,2,3,4]
	143 per 1000	89 per 1000 (34 to 223)			
Recurrence of ROP by 55 weeks' PMA	Study population		RR 0.29 (0.12 to 0.7)	76 (1 RCT)	⊕⊕◯◯ LOW [1,3]
	500 per 1000	145 per 1000 (60 to 350)			

*Only the outcomes for which data are available are reported here;
#The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

[1]Outcome assessment not blinded

[2]Serious risk of bias in analysis (unit of analysis error)

[3]Unclear risk of selection bias

[4]95% CI around the pooled estimate includes both 1) no effect and 2) appreciable benefit or appreciable harm

DISCUSSION

Summary of main results

The systematic review included three studies, of which two evaluated the effects of bevacizumab monotherapy while the third examined the effects of intravitreal pegaptanib *plus* laser therapy. When used as monotherapy, intravitreal bevacizumab did not improve short-term structural outcomes (partial or complete retinal detachment) but significantly reduced the risk of refractive errors at 30 months of age. When used in conjunction with laser therapy, intravitreal pegaptanib reduced the risk of retinal detachment in infants with stage 3+ ROP. Both the agents reduced the risk of recurrence of ROP requiring re-treatment in the first few weeks of life. No significant difference was noted in the incidence of local adverse events with either of the drugs. On subgroup analysis, the risk of all the outcomes - except for recurrence of ROP requiring re-treatment - was found to be similar in infants with zone I ROP and those with zone II posterior ROP receiving intravitreal bevacizumab monotherapy. However, the numbers were too small to draw any meaningful conclusion on the subgroup analysis.

Overall completeness and applicability of evidence

The currently available evidence is 'incomplete' for three major reasons. Firstly, the limited number of studies included in the review. Despite the well established pathophysiological rationale for using anti-VEGF agents and the short-term benefits observed in numerous case reports and case series (Shah 2007; Kong 2008; Mintz-Hittner 2008), only three RCTs have been published so far. Secondly, the long-term beneficial effects, if any, in terms of favourable structural and functional outcomes are not known. Both the agents were found to reduce the risk of recurrent ROP requiring re-treatment in the first few weeks of life but it is not clear if this benefit would translate into lesser risk of blindness, severe visual impairment or unfavourable structural outcomes at a later age. Also, the risk of recurrence of ROP beyond the first few weeks of life in the intervention group is not known. Thirdly, the safety concerns of anti-VEGF drugs are yet to be addressed. One trial (BEAT-ROP Trial 2011) reported no difference in the risk of mortality between the intervention and control groups at a mean age of 30 months but the number of events was very small. Given the potential risk of systemic absorption and consequent adverse effects like cerebrovascular accidents following intravitreal anti-VEGF therapy, the lack of evidence on safety outcomes is a major concern.

The incomplete evidence is indeed a serious dampener in the quest to identify a simple, safe, and effective therapy for ROP. Unlike the current standard of therapy, laser or cryotherapy, anti-VEGF administration is technically simple and does not require general anaesthesia or the services of a skilled retinal surgeon. This could be a great boon, particularly in settings with limited resources from low- and middle-income countries. There is an urgent need to generate more evidence on the long-term structural outcomes as well as the adverse effects following intravitreal therapy with anti-VEGF agents before they can be considered for routine clinical use in infants with ROP. Future studies should also examine how these drugs affect the natural history of the disease, the focus being late recurrence that might warrant long-term follow-up and the risk of local complications like infections following therapy, especially in resource-restricted settings.

Quality of the evidence

We intended to include all the outcomes - primary and secondary - of the review in the 'Summary of findings' tables. However, many of the outcomes were not reported in the included studies. Therefore, only seven outcomes for the comparison of 'Anti-VEGF vs cryo/laser therapy' and three outcomes for the comparison of 'Anti-VEGF plus cryo/laser therapy vs cryo/laser therapy alone' were reported in the 'Summary of findings' tables.

The quality of evidence was graded as very low to low for almost all outcomes of the review (Summary of findings for the main comparison; Summary of findings 2). The risk of detection bias was high in all the studies because the outcome assessors were not blinded to the group allocation. The risk of other biases including selection bias and reporting bias was low in BEAT-ROP Trial 2011; the risk of these biases is not clear in the other two studies (Autrata 2012; Lepore 2014).

Potential biases in the review process

Most outcomes of the review were not reported in the included studies. The authors of the studies were contacted to collect additional information on these outcomes but no response was obtained.

Agreements and disagreements with other studies or reviews

The earlier systematic review *Off-label use of bevacizumab for severe retinopathy of prematurity*, was published in 2009 (Micieli 2009). It included nine articles, of which six were case reports, two were retrospective studies, and one was a prospective case series. It found considerable variability in how bevacizumab is used for the treatment of ROP, and concluded that "further randomized control trials are warranted". The current review included three randomized trials - two on intravitreal bevacizumab monotherapy and one on intravitreal pegaptanib combination therapy.

AUTHORS' CONCLUSIONS

Implications for practice

Intravitreal bevacizumab reduces the risk of refractive errors during childhood when used as monotherapy while intravitreal pegaptanib reduces the risk of retinal detachment when used in conjunction with laser therapy in infants with type 1 ROP. Quality of evidence was, however, low for both these outcomes because of the risk of detection and other biases. Effects on other critical outcomes and, more importantly, the long-term systemic adverse effects of the drugs are not known. The insufficient data precludes strong conclusions favouring routine use of intravitreal anti-VEGF agents in preterm infants with type 1 ROP.

Implications for research

Further randomized controlled trials are needed to evaluate the effect of anti-VEGF agents, when used as monotherapy or as a part of combination therapy with laser/cryotherapy, on (i) structural and functional outcomes in childhood and (ii) delayed systemic adverse effects such as stroke, myocardial dysfunction, and adverse neurodevelopmental outcomes in preterm infants with severe ROP. Ideally, the trials should be large multi-centre studies with adequate sample size to detect a clinically important difference in the risk of one or more of the delayed systemic adverse effects. An attempt should be made to ensure blinding of caregivers and outcome assessors to the group allocation in these trials.

REFERENCES

References to studies included in this review

Autrata 2012 *{published data only (unpublished sought but not used)}*
Autrata R, Krejčířová I, Šenková K, Holoušová M, Doležel Z, Borek I. Intravitreal pegaptanib combined with diode laser therapy for stage 3+ retinopathy of prematurity in zone I and posterior zone II. *European Journal of Ophthalmology* 2012;**22**(5):687–94. [PUBMED: 22669848]

BEAT-ROP Trial 2011 *{published data only (unpublished sought but not used)}*
Geloneck MM, Chuang AZ, Clark WL, Hunt MG, Norman AA, Packwood EA, et al. BEAT-ROP Cooperative Group. Refractive outcomes following bevacizumab monotherapy compared with conventional laser treatment: a randomized clinical trial. *JAMA Ophthalmology* 2014;**132**(11):1327–33. [PUBMED: 25103848]
* Mintz-Hittner HA, Kennedy KA, Chuang AZ, BEAT-ROP Cooperative Group. Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. *New England Journal of Medicine* 2011;**364**(7):603–15. [PUBMED: 21323540]
NCT00622726. Bevacizumab Eliminates the Angiogenic Threat for Retinopathy of Prematurity (BEAT-ROP). https://clinicaltrials.gov/ct2/show/NCT00622726 (accessed 10 January 2016).

Lepore 2014 *{published data only}*
Lepore D, Quinn GE, Molle F, Baldascino A, Orazi L, Sammartino M, et al. Intravitreal bevacizumab versus laser treatment in type 1 retinopathy of prematurity: report on fluorescein angiographic findings. *Ophthalmology* 2014;**121**(11):2212–9. [PUBMED: 25001158]

References to studies awaiting assessment

Kong 2015 *{published data only}*
Kong L, Bhatt AR, Demny AB, Coats DK, Li A, Rahman EZ, et al. Pharmacokinetics of bevacizumab and its effects on serum VEGF and IGF-1 in infants with retinopathy of prematurity. *Investigative Ophthalmology & Visual Science* 2015;**56**(2):956–61. [DOI: 10.1167/iovs.14-15842; PUBMED: 25613938]

Moran 2014 *{published data only}*
Moran S, O'Keefe M, Hartnett C, Lanigan B, Murphy J, Donoghue V. Bevacizumab versus diode laser in stage 3 posterior retinopathy of prematurity. Acta Ophthalmologica 2014; Vol. 92, issue 6:e496–7. [PUBMED: 24428792]

Additional references

Aiello 1995
Aiello LP, Pierce EA, Foley ED, Takagi H, Chen H, Riddle L, et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proceedings of the National Academy of Sciences of the United States of America* 1995;**92**(23):10457–61. [PUBMED: 7479819]

American Academy of Pediatrics 2006
Section on Ophthalmology American Academy of Pediatrics, American Academy of Ophthalmology, American Association for Pediatric Ophthalmology and Strabismus.

Screening examination of premature infants for retinopathy of prematurity. *Pediatrics* 2006;**117**(2):572–6. [PUBMED: 16452383]

Andersen 1999

Andersen C, Phelps D. Peripheral retinal ablation for threshold retinopathy of prematurity in preterm infants. *Cochrane Database of Systematic Reviews* 1999, Issue 3. [DOI: 10.1002/14651858.CD001693]

Ashton 1953

Ashton N, Ward B, Serpell G. Role of oxygen in the genesis of retrolental fibroplasia; a preliminary report. *British Journal of Ophthalmology* 1953;**37**(9):513–20. [PUBMED: 13081949]

Ashton 1954

Ashton N, Ward B, Serpell G. Effect of oxygen on developing retinal vessels with particular reference to the problem of retrolental fibroplasia. *British Journal of Ophthalmology* 1954;**38**(7):397–432. [PUBMED: 13172417]

Committee for Classification of ROP 1984

The Committee for the Classification of Retinopathy of Prematurity. An international classification of retinopathy of prematurity. *Archives of Ophthalmology* 1984;**102**(8): 1130–4. [PUBMED: 6547831]

Deeks 2011

Deeks JJ, Higgins JPT, Altman DG (editors). Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

ET-ROP Group 2003

Early Treatment For Retinopathy Of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Archives of Ophthalmology* 2003;**121**(12):1684–94. [PUBMED: 14662586]

Gilbert 2005

Gilbert C, Fielder A, Gordillo L, Quinn G, Semiglia R, Visintin P, et al. Characteristics of infants with severe retinopathy of prematurity in countries with low, moderate, and high levels of development: implications for screening programs. *Pediatrics* 2005;**115**(5):e518–25. [PUBMED: 15805336]

Gilbert 2008

Gilbert C, Muhit M. Twenty years of childhood blindness: what have we learnt?. *Community Eye Health / International Centre for Eye Health* 2008;**21**(67):46–7.

GRADEpro GDT [Computer program]

McMaster University (developed by Evidence Prime, Inc.). GRADEpro GDT: GRADEpro Guideline Development Tool. McMaster University (developed by Evidence Prime, Inc.), 2015.

Higgins 2003

Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**: 557–60.

Higgins 2011

Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Higgins 2011a

Higgins JPT, Altman DG, Sterne JAC (editors). Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Higgins 2011b

Higgins JPT, Deeks JJ, Altman DG (editors). Chapter 16: Special topics in statistics. In: Higgins JPT, Green S (editors), *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Kong 2008

Kong L, Mintz-Hittner HA, Penland RL, Kretzer FL, Chevez-Barrios P. Intravitreous bevacizumab as anti-vascular endothelial growth factor therapy for retinopathy of prematurity: a morphologic study. *Archives of Ophthalmology* 2008;**126**(8):1161–3. [PUBMED: 18695118]

Lashkari 2000

Lashkari K, Hirose T, Yazdany J, McMeel JW, Kazlauskas A, Rahimi N. Vascular endothelial growth factor and hepatocyte growth factor levels are differentially elevated in patients with advanced retinopathy of prematurity. *The American Journal of Pathology* 2000;**156**(4):1337–44. [PUBMED: 10751359]

Law 2010

Law JC, Recchia FM, Morrison DG, Donahue SP, Estes RL. Intravitreal bevacizumab as adjunctive treatment for retinopathy of prematurity. *Journal of AAPOS* 2010;**14**(1): 6–10. [PUBMED: 20227614]

Mantagos 2009

Mantagos IS, Vanderveen DK, Smith LE. Emerging treatments for retinopathy of prematurity. *Seminars in Ophthalmology* 2009;**24**(2):82–6. [PUBMED: 19373691]

Micieli 2009

Micieli JA, Surkont M, Smith AF. A systematic analysis of the off-label use of bevacizumab for severe retinopathy of prematurity. *American Journal of Ophthalmology* 2009;**148** (4):536–543.e2. [PUBMED: 19660736]

Mintz-Hittner 2008

Mintz-Hittner HA, Kuffel RR Jr. Intravitreal injection of bevacizumab (avastin) for treatment of stage 3 retinopathy

of prematurity in zone I or posterior zone II. *Retina* 2008;
28(6):831–8. [PUBMED: 18536599]

Palmer 1997

Palmer EA. [What have we learned about retinopathy of prematurity during the past ten years? Progress in retinopathy of prematurity]. Proceedings of the International Symposium on Retinopathy of Prematurity, 1997, Taormina, Italy. Amsterdam/New York: Kugler Publications, 1997.

RevMan 2014 [Computer program]

The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Schünemann 2011

Schünemann HJ, Oxman AD, Vist GE, Higgins JPT, Deeks JJ, Glasziou P, et al. Chapter 12: Interpreting results and drawing conclusions. In: Higgins JPT, Green S (editors), *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Shah 2007

Shah PK, Narendran V, Tawansy KA, Raghuram A, Narendran K. Intravitreal bevacizumab (Avastin) for post laser anterior segment ischemia in aggressive posterior

retinopathy of prematurity. *Indian Journal of Ophthalmology* 2007;**55**(1):75–6. [PUBMED: 17189897]

Smith 2003

Smith LE. Pathogenesis of retinopathy of prematurity. *Seminars in Neonatology* 2003;**8**(6):469–73. [PUBMED: 15001119]

Sterne 2011

Sterne JAC, Egger M, Moher D (editors). Chapter 10: Addressing reporting biases. In: Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Intervention*. Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Tasman 2006

Tasman W, Patz A, McNamara JA, Kaiser RS, Trese MT, Smith BT. Retinopathy of prematurity: the life of a lifetime disease. *American Journal of Ophthalmology* 2006;**141**(1): 167–74. [PUBMED: 16386993]

Ueta 2009

Ueta T, Yanagi Y, Tamaki Y, Yamaguchi T. Cerebrovascular accidents in ranibizumab. Ophthalmology 2009; Vol. 116, issue 2:362. [PUBMED: 19187826]

Wong 2015

Wong RK, Hubschman S, Tsui I. Reactivation of retinopathy of prematurity after ranibizumab treatment. *Retina* 2015;**35**(4):675–80.

* *Indicates the major publication for the study*

C H A R A C T E R I S T I C S   O F   S T U D I E S

Characteristics of included studies *[ordered by study ID]*

Autrata 2012

Methods	Randomised controlled trial
Participants	Preterm infants with stage 3+ ROP in Zone I or posterior zone II (n = 76); Single centre, university hospital, Brno - Czech republic
Interventions	Intervention: intravitreal pegaptanib (0.3 mg in 0.02 ml of solution) combined with confluent laser therapy Control: conventional laser therapy
Outcomes	Primary: treatment success defined as absence of recurrence of stage 3+ ROP in one or both eyes by 55 weeks' postmenstrual age Secondary: time of regression and decrease of plus signs, development of peripheral retinal vessels after treatment, final structural/anatomic outcomes
Notes	We tried to contact the authors for additional information on methods and other outcomes (relevant to the review) but did not get any response

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	How the random sequence was generated is not mentioned
Allocation concealment (selection bias)	Unclear risk	Not clear if the random allocation was concealed
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Not clear if the clinical team was masked to the intervention group
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not clear if the outcome assessors were blinded to the group allocation
Incomplete outcome data (attrition bias) All outcomes	Low risk	No loss to follow-up
Selective reporting (reporting bias)	Unclear risk	Not clear if all the outcomes were reported

BEAT-ROP Trial 2011

Methods	Randomised controlled trial
Participants	Infants with birth weight 1500 g or less and gestational age of 30 weeks or less with stage 3+ ROP in zone I or zone II posterior in each eye (n = 150) Multi-centre trial conducted at 15 hospitals in the United States of America
Interventions	Intervention: intravitreal bevacizumab monotherapy (0.625 mg in 0.025 ml of solution) Control: conventional laser therapy
Outcomes	Primary: treatment failure defined as recurrence of neovascularisation in one or both eyes and requiring re-treatment by 54 weeks' postmenstrual age Secondary: structural outcomes of recurrence (macular dragging, retinal detachment), complications requiring intraocular surgery (cornea opacity requiring corneal transplant, lens opacity requiring cataract removal), mortality
Notes	We tried to contact the authors for additional information on other outcomes relevant to the review but did not get any response

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Secure computer-generated randomisation schedule stratified on the basis of zone by a study group member who did not participate in enrolment
Allocation concealment (selection bias)	Low risk	Treatment assignments were revealed to the investigators only after the eligibility for enrolment had been confirmed
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study was controlled but not masked owing to the marks made by the laser therapy
Blinding of outcome assessment (detection bias) All outcomes	High risk	Retcam photographs to document recurrence by treating and confirming ophthalmologists without masking of treatment assignments, before deciding on additional treatment Unmasked practicing paediatric ophthalmologists performed the cycloplegic retinoscopic refractions to assess the refractive errors at 30 months of age
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcome data of all infants who survived until 54 weeks' postmenstrual age were included in the analysis; about 17% of eligible infants were lost to follow-up at 30 months of age (refractive outcomes)
Selective reporting (reporting bias)	Low risk	All outcomes mentioned in the protocol were reported

BEAT-ROP Trial 2011   *(Continued)*

Other bias	Low risk	-

Lepore 2014

Methods	Randomised controlled trial Randomised one eye of enrolled infants to conventional laser and the other eye to bevacizumab
Participants	Infants with type 1 ROP in zone I in both eyes and required treatment, according to ET-ROP criteria (n = 13)
Interventions	'Intervention' eye: intravitreal bevacizumab monotherapy (0.5 mg in 0.02 ml of balanced salt solution) 'Control' eye: conventional laser therapy
Outcomes	Abnormailities on fluorescein angiography (FA) - macular abnormalities (absence of foveolar avascular zone or hyperfluorescent lesion), capillary bed loss, linear choroidal filling pattern; complete retinal detachment (stage 5); mortality at three months of age
Notes	The eye assigned to conventional laser peripheral ablation was treated first. The eye randomized to receive bevacizumab was then prepared and 0.5 mg (0.02 ml) of bevacizumab was injected intravitreally

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "... randomly selected using a random number series"
Allocation concealment (selection bias)	Unclear risk	Not clear if the random allocation was concealed
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not possible because one eye is randomized to intervention while the other eye to control (laser)
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Not clear if the outcome assessors were blinded to the group allocation
Incomplete outcome data (attrition bias) All outcomes	Low risk	No loss to follow-up
Selective reporting (reporting bias)	Unclear risk	Not clear if all the outcomes were reported (secondary outcomes not available in the published protocol)

Lepore 2014   (*Continued*)

Other bias	High risk	Because eyes were randomized, the eye randomized to control group would have been exposed to both anti-VEGF agents and control treatment resulting in better outcomes if there was significant systemic absorption of bevacizumab

Characteristics of studies awaiting assessment   *[ordered by study ID]*

Kong 2015

Methods	Randomised controlled trial
Participants	Twenty-four infants with type 1 ROP
Interventions	Twenty-four infants with type 1 ROP were randomized into three treatment groups: intravitreal injection of bevacizumab (IVB) at 0.625 mg per eye per dose, IVB at 0.25 mg per eye per dose, and laser
Outcomes	Blood samples were collected prior to treatment and on post-treatment days 2, 14, 42, and 60. Weekly body weights were documented from birth until 60 days post treatment. Serum levels of bevacizumab, free VEGF, and IGF-1 were measured with enzyme-linked immunosorbent assay (ELISA)
Notes	

Moran 2014

Methods	Prospective case-control study
Participants	Fourteen infants with symmetrical zone 1 or posterior zone 2 Stage 3 + ROP
Interventions	Comparing intravitreal bevacizumab in one eye to laser therapy in the fellow eye
Outcomes	"We observed rapid regression of ROP in all eyes injected with bevacizumab, as well as resolution of plus disease and flattening of the ridge by 48 hr post-injection in all eyes. Further vascularization was noted with complete regression taking up to sixty weeks in some eyes In our study, four of 14 eyes (28.6%) had recurrence of ROP; three eyes (21.42%) which had bevacizumab treatment and one eye (7.14%) with conventional laser therapy. There was a significant time delay to recurrence in the bevacizumab group compared with laser, with a mean age of 51 weeks PMA at time of recurrence in bevacizumab-treated eyes compared with 37 weeks PMA in the laser-treated eye." Moran 2014
Notes	

DATA AND ANALYSES

Comparison 1. Anti-VEGF vs cryo/laser therapy

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Structural outcome - partial or complete retinal detachment	1	143	Risk Ratio (M-H, Fixed, 95% CI)	1.04 [0.21, 5.13]
1.1 Zone I	1	64	Risk Ratio (M-H, Fixed, 95% CI)	0.21 [0.01, 4.26]
1.2 Zone II posterior	1	79	Risk Ratio (M-H, Fixed, 95% CI)	5.13 [0.25, 103.45]
2 Structural outcome - complete retinal detachment (unit of analysis: eyes)	1	26	Risk Ratio (M-H, Fixed, 95% CI)	0.33 [0.01, 7.50]
3 Refractive error - very high myopia - at 30 months of age (unit of analysis: eyes)	1	211	Risk Ratio (M-H, Fixed, 95% CI)	0.06 [0.02, 0.20]
3.1 Zone I	1	87	Risk Ratio (M-H, Fixed, 95% CI)	0.07 [0.02, 0.30]
3.2 Zone II posterior	1	124	Risk Ratio (M-H, Fixed, 95% CI)	0.05 [0.01, 0.34]
4 Refractive error - spherical equivalent refractions - at 30 months of age (unit of analysis: eyes)	1	211	Mean Difference (IV, Fixed, 95% CI)	5.68 [4.33, 7.02]
4.1 Zone I	1	87	Mean Difference (IV, Fixed, 95% CI)	6.93 [4.26, 9.60]
4.2 Zone II posterior	1	124	Mean Difference (IV, Fixed, 95% CI)	5.25 [3.69, 6.81]
5 Mortality before discharge from primary hospital	1	150	Risk Ratio (M-H, Fixed, 95% CI)	1.50 [0.26, 8.75]
5.1 Zone I	1	67	Risk Ratio (M-H, Fixed, 95% CI)	1.03 [0.07, 15.80]
5.2 Zone II posterior	1	83	Risk Ratio (M-H, Fixed, 95% CI)	1.95 [0.18, 20.71]
6 Mortality at 30 months of age	1	150	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.30, 2.45]
6.1 Zone I	1	67	Risk Ratio (M-H, Fixed, 95% CI)	0.62 [0.16, 2.38]
6.2 Zone II posterior	1	83	Risk Ratio (M-H, Fixed, 95% CI)	1.46 [0.26, 8.31]
7 Local adverse effects - corneal opacity requiring corneal transplant (unit of analysis: eyes)	1	286	Risk Ratio (M-H, Fixed, 95% CI)	0.34 [0.01, 8.26]
7.1 Zone I	1	128	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.2 Zone II posterior	1	158	Risk Ratio (M-H, Fixed, 95% CI)	0.34 [0.01, 8.26]
8 Local adverse effects - lens opacity requiring cataract removal (unit of analysis: eyes)	1	286	Risk Ratio (M-H, Fixed, 95% CI)	0.15 [0.01, 2.79]
8.1 Zone I	1	128	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
8.2 Zone II posterior	1	158	Risk Ratio (M-H, Fixed, 95% CI)	0.15 [0.01, 2.79]
9 Recurrence of ROP by 54 weeks PMA	1	143	Risk Ratio (M-H, Fixed, 95% CI)	0.22 [0.08, 0.62]
9.1 Zone I	1	64	Risk Ratio (M-H, Fixed, 95% CI)	0.15 [0.04, 0.62]
9.2 Zone II posterior	1	79	Risk Ratio (M-H, Fixed, 95% CI)	0.41 [0.08, 1.99]

Comparison 2.   Anti-VEGF plus cryo/laser therapy vs cryo/laser therapy

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Structural outcome - retinal detachment (unit of analysis: eyes)	1	152	Risk Ratio (M-H, Fixed, 95% CI)	0.26 [0.12, 0.55]
2 Local adverse effects - perioperative retinal haemorrhages (unit of analysis: eyes)	1	152	Risk Ratio (M-H, Fixed, 95% CI)	0.62 [0.24, 1.56]
3 Recurrence of ROP by 55 weeks PMA	1	76	Risk Ratio (M-H, Fixed, 95% CI)	0.29 [0.12, 0.70]

Comments from Zhou et al, 1 April 2016

Summary

"The authors mentioned that several safety concerns, such as cerebrovascular accidents following intravitreal anti-VEGF therapy, would be examined. However, the impact of anti-VEGF on choroid (the sole blood supply for the macula) is not fully discussed. A number of recent reports have demonstrated choroidal thinning in patients with a history of ROP (1-3) as well as in a well-established rodent model of ROP (4). Physiologically, VEGF plays a critical role for choroidal vascular development (5, 6). Mice lacking VEGF-120 and VEGF-164, major VEGF isoforms secreted by retinal pigment epithelium (RPE), experienced progressive choroidal dengeration (5). Meanwhile, a study on macaques showed that intravitreal bevacizumab (IVB) was able to reach choroid; and the drug was found to be concentrated in photoreceptors and choroidal endothelium (7). Lastly, serum VEGF levels have been found to be suppressed for two weeks after ROP patients received IVB treatment (8, 9), suggesting anti-VEGF is able to penetrate retinal-blood barrier.

Indeed, adverse effects of IVB among ROP patients have been documented, including choroidal ruptures ten weeks after injection (10) and choroidal ischemia in a neonate after a single, bilateral IVB injection (11). Since anti-VEGF has a direct effect on vasculature, we feel that a thorough review about anti-VEGF's impact on choroid, such as linear choroidal filling pattern (mentioned in the review) and choroidal thickness (readily measured by optical coherent tomography), is warranted."

References
1. Anderson MF, Ramasamy B, Lythgoe DT, Clark D. Choroidal thickness in regressed retinopathy of prematurity. Eye (Lond) 2014; 28(12):1461-8.
2. Erol MK, Coban DT, Ozdemir O, Dogan B, Tunay ZO, Bulut M. Choroidal thickness in infants with retinopathy of prematurity. Retina 2015;36(6):1191-8.
3. Park KA, Oh SY. Analysis of spectral-domain optical coherence tomography in preterm children: retinal layer thickness and choroidal thickness profiles. Investigative Ophthalmology & Visual Science 2012;53(11):7201-7.
4. Shao Z, Dorfman AL, Seshadri S, Djavari M, Kermorvant-Duchemin E, Sennlaub F, et al. Choroidal involution is a key component of oxygen-induced retinopathy. Investigative Ophthalmology & Visual Science 2011;52(9):6238-48.
5. Saint-Geniez M, Kurihara T, Sekiyama E, Maldonado AE, D'Amore PA. An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. Proceedings of the National Academy of Sciences of the United States of America 2009;106(44): 18751-6.
6. Zhu M, Bai Y, Zheng L, Le YZ. Presence of RPE-produced VEGF in a timely manner is critical to choroidal vascular development. Advances in Experimental Medicine and Biology 2012;723:299-304.
7. Heiduschka P, Fietz H, Hofmeister S, Schultheiss S, Mack AF, Peters S, et al. Penetration of bevacizumab through the retina after intravitreal injection in the monkey. Investigative Ophthalmology & Visual Science 2007;48(6):2814-23.
8. Wu WC, Lien R, Liao PJ, Wang NK, Chen YP, Chao AN, et al. Serum levels of vascular endothelial growth factor and related factors after intravitreous bevacizumab injection for retinopathy of prematurity. JAMA Ophthalmology 2015;133(4):391-7.
9. Sato T, Wada K, Arahori H, Kuno N, Imoto K, Iwahashi-Shima C, et al. Serum concentrations of bevacizumab (avastin) and vascular endothelial growth factor in infants with retinopathy of prematurity. American Journal of Ophthalmology 2012;153(2):327-33.e1.
10. Atchaneeyasakul LO, Trinavarat A. Choroidal ruptures after adjuvant intravitreal injection of bevacizumab for aggressive posterior retinopathy of prematurity. Journal of Perinatology 2010;30(7):497-9.
11. Chhablani J, Rani PK, Balakrishnan D, Jalali S. Unusual adverse choroidal reaction to intravitreal bevacizumab in aggressive posterior retinopathy of prematurity: the Indian Twin Cities ROP screening (ITCROPS) data base report number 7. Seminars Ophthalmology 2014;29(4):222-5.

Reply

"We sincerely thank Zhou et al for their insightful comments on our review "Anti-vascular endothelial growth factor (VEGF) drugs for treatment of retinopathy of prematurity".

We do agree with them regarding the potential risk of choroidal thinning and ischemia following anti-VEGF therapy. Recent reports indicate that the choroidal thickness is inversely related to the severity of ROP.[2] There is a risk that anti-VEGF therapy might further aggravate this phenomenon. Our current protocol or review did not include these outcomes. We shall enlist the following two outcomes, namely:

- median thickness of the choroid and
- incidence of choroidal ischemia/rupture

under 'local adverse effects' in the next update of our review." Review update publication is planned for 2017.

1. Erol MK, Coban DT, Ozdemir O, Dogan B, Tunay ZO, Bulut M. Choroidal thickness in infants with retinopathy of prematurity. Retina. 2015 Nov 18. [Epub ahead of print] PubMed PMID: 26583308.

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WHAT'S NEW

Last assessed as up-to-date: 1 January 2016.

Date	Event	Description
25 May 2016	Amended	Review comment and author response added. Comments to be included in the next review update scheduled for 2017

CONTRIBUTIONS OF AUTHORS

MJS, JS, and MM searched the literature with the help of the Cochrane Neonatal Review Group trials search co-ordinator. MJS, JS and MM independently extracted data and assessed included studies for risk of bias. MJS conducted the data analysis and wrote the final draft with inputs from the remaining authors.

DECLARATIONS OF INTEREST

Mari Jeeva Sankar: no conflict of interest

Jhuma Sankar: no conflict of interest

Manisha Mehta: no conflict of interest

Vishnu Bhat: no conflict of interest

Renuka Srinivasan: no conflict of interest.

SOURCES OF SUPPORT

Internal sources

External sources

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The differences between the protocol and review are listed below:

a. Secondary outcomes

Added: One additional outcome - recurrence of ROP requiring re-treatment by 54-55 weeks PMA - that was not planned in the protocol.

b. Dealing with missing data

Deleted: *"For dichotomous data, if drop-outs exceed 10% for any trial, we will assign the worse outcomes to those who were lost to follow-up and assess the impact in the study results in sensitivity analyses (Higgins 2011)."*

c. Assessment of heterogeneity

Deleted: *"If statistical heterogeneity is detected, we will explore the possible causes. We intend to use the fixed-effect model if the I2 statistic is less than 60%; in the event that the I2 is more than 60%, we will use the random effects."*

d. Data synthesis

Deleted: *"For ordinal outcomes (as in Likert scale for parental satisfaction), we will summarize the data using methods for continuous variables - as a difference in means or standardized difference in means. Depending upon the heterogeneity, we plan to use either fixed-effect or random effects models with inverse variance weighting for the meta-analyses."*

e. Measures of treatment effect

Deleted: *"We used the fixed-effect model for pooling the results of individual studies."*

f. Unit of analysis issues

Added: *"However, if a given study had randomized eyes and not infants, we intended to use the study data but refrained from pooling it with data of studies that had randomized infants. We a priori decided to use the eye-level data (and not infant-level data) in these studies, i.e. incidence of outcomes in eyes randomized to anti-VEGF vs. incidence of outcomes in eyes randomized to the control group; consequently, individual-level outcomes such as mortality were not considered in these studies. We a priori assumed that the beneficial effect, if any, would be diluted in them, i.e. the effect size would be closer to the null effect, if systemic absorption of anti-VEGF agents were to occur (because the eye randomized to control group would be exposed to both anti-VEGF agents and 'control' treatment).*

If a given study had randomized infants but provided the outcome data for eyes, we planned to contact the authors to obtain infant-level data so as to avoid unit of analysis error - using eyes as the denominator without adjusting for non-independence between the eyes may result in spuriously precise results, i.e. narrow confidence intervals (similar to that seen in cluster randomized trials when the clusters are randomized but the outcomes are analysed at the individual level without adjusting for 'cluster' effect (Higgins 2011). If that information could not be obtained, we used the data for eyes but mentioning upfront that the analysis refers to eyes and not the infants."

INDEX TERMS

Medical Subject Headings (MeSH)

Angiogenesis Inhibitors [*administration & dosage]; Aptamers, Nucleotide [*administration & dosage]; Bevacizumab [*administration & dosage]; Combined Modality Therapy; Cryotherapy [methods]; Intravitreal Injections; Laser Therapy [methods]; Randomized Controlled Trials as Topic; Retinal Detachment [prevention & control]; Retinopathy of Prematurity [*drug therapy]; Vascular Endothelial Growth Factor A [*antagonists & inhibitors]

MeSH check words

Humans; Infant, Newborn