Retinopathy of Prematurity: Two Distinct Mechanisms That Underlie Zone 1 and Zone 2 Disease

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• PURPOSE: **In its most severe form, retinopathy of prematurity (ROP) is located in posterior retina and affects the smallest, most premature infants. We hypothesize that, depending on whether vasculogenesis (de novo formation of new vessels by transformation of vascular precursor cells (VPCs)) or angiogenesis (budding from existing vessels) is perturbed, it results in significant differences in clinical presentation and therapeutic outcome observed in zone 1 vs zone 2 ROP.**

• DESIGN: **The study is a retrospective analysis of the difference in outcome between zones 1 and 2 ROP after cryotherapy and laser therapy.**

• METHODS: **A review of the clinical presentation of zones 1 and 2 ROP that correlate this with the topography of formation of human retinal vasculature through vasculogenesis and angiogenesis.**

• RESULTS: **Population data on susceptible infants, and outcome statistics of clinical trials are given. Digital images show a correlation between ROP in zone 1 with the region of the retina vascularized through vasculogenesis.**

• CONCLUSION: **Zone 1 ROP is correlated with vessel development by vasculogenesis, relative insensitivity to laser/cryotherapy and poorer anatomic and visual outcomes. This suggests that, if the vasculogenic process is perturbed, it results in a distinct clinical presentation, poorer response to therapy, and poorer visual outcome. When the current international classification was developed, knowledge of the processes of human retinal vascular development was incomplete. The work presented here provides a framework for the development of a modification to incorporate these ideas without sacrifice of the essential elements of the international classification of ROP. (Am J Ophthalmol 2006;142:46–59. © 2006 by Elsevier Inc. All rights reserved.)**

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RETINOPATHY OF PREMATURITY (ROP) IS A DISEASE of developing retinal blood vessels. It is classified according to location, extent, and stage of the disease that are observed on indirect ophthalmoscopy[1,2] In its most severe form, ROP is located in the posterior retina and involves the entire circumference of the developing vasculature. Our recent studies of retinal vascular development have demonstrated two phases. An early phase, vasculogenesis, occurs where vascular precursor cells (VPCs) of mesenchymal origin exit from the optic nerve and are responsible for the formation of primordial vessels of the central superficial plexus, which includes the four major arcades in the posterior retina. The process begins before 14 weeks of gestation (WG) and is complete by 21 WG.[3] The later phase, angiogenesis, the formation of new vessels through budding from existing vessels is responsible for increasing capillary density of the central retina and forming the peripheral vessels of the superficial plexus and deep capillary plexus and the peripapillary radial capillaries.[3]

Our hypothesis was that the reason for the poor results of ablative therapy in zone 1 ROP is that the vessels in zone 1 are of vasculogenic origin and are insensitive to vascular endothelial growth factor 7–165 ($VEGF_{165}$) modulation and hence to the effect of laser or cryotherapy. In support of this hypothesis, three lines of evidence are presented: The first line of evidence is in support of the contribution of vasculogenesis and angiogenesis to the formation of the human retinal vasculature. Building on this anatomic framework, the second line of evidence is data from existing randomized trials that clearly demonstrate a difference of outcome among three trials. Emphasis is placed on the response of zone 1 ROP to laser or cryotherapy that was applied in randomized clinical trials,[4–6] where such outcome figures are obtainable. As the third line of evidence, we present the clinical picture of zone 1 ROP and contrast its appearance with the milder disease in

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zone 2. Included in this description are the characteristics of the infants in whom it occurs and the location, extent, and severity of the disease that is located in zone 1. The final aim was to discuss the implications of our hypothesis as an explanation for the discrepancy between the failure rates of therapy in zones 1 and 2, for the classification of ROP, and finally for the timing of the injury-producing ROP during the course of retinal vascular development.

Our earlier studies showed that, in the human, retinal vascularization begins before 14 WG and is complete by approximately 36 to 40 WG.[3] Studies of human fetal retinae have shown that vascularization results from two distinct processes that are different in time and location in their role in vascularizing the human fetal retina.[3,7,8] Vasculogenesis involves the invasion of VPCs from the optic nerve head and their proliferation, differentiation, and coalescence into tubes to form the primordial vascular arcades of the superficial vascular plexus that is centered at the optic nerve head.[3] Spindle-shaped (fusiform or tapering at each end) adenosine diphosphatase (ADPase) and VPC invade the retina from the optic nerve head before 14 WG and are no longer apparent by 21 WG.[3,6,8] ADPase is an ectoenzyme on the luminal surface of endothelial cells that is responsible for degrading extracellular ADP and preventing platelet aggregation. This primitive network is expanded through angiogenesis, by sprouting, bridging, and branching and by introsusception of pre-existing vessels. Angiogenesis takes over when vasculogenesis is completed and ends when the superficial and deeper retinal plexus have reached the ora serrata. Thus, vasculogenesis creates the major arcades de novo, and angiogenesis completes the formation of all remaining retinal vessels. Whereas angiogenesis appears to be driven by hypoxia-induced $VEGF_{165}$,[3,9] vasculogenesis in the human retina is independent of metabolic demand and hypoxia-induced $VEGF_{165}$ expression.[3] Evidence for this conclusion stems from the observation that substantial vascularization of the human retina occurs in the absence of $VEGF_{165}$ expression and the fact that vasculogenesis is complete before the differentiation of most retinal neurons. Further evidence of the independence of vasculogenesis from $VEGF_{165}$ is provided by $VEGF_{165}$ knockout mice, where vessels still form by vasculogenesis but are highly abnormal.

During formation of the human retinal vasculature, neuronal elements, microglia (including astrocytes and Müller cells)[9,10] immune and phagocytic cells (including retinal microglia and macrophages)[11] and vasoformative elements (perivascular cells and vascular endothelial cells, pericytes and smooth muscle cells)[12] interact in complex ways that result in the formation of a vascular tree that is well-matched to the metabolic needs of the tissue.[13,14]

This study was conducted under the guidelines established by the institutional review board at Columbia University and the Human Ethics Committee at the University of Sydney.

TABLE 1. Incidence of Survival of Micropremature Per 1000 Live Births in the United States[15]*

Birth Weight (g)	1990	1995	2002
<500	105	97	138
500–749	364	472	510
750–1000	743	818	845

*Data provided by Martin J, and Matthews T, National Center for Health Care Statistics, CDC, Hyattsville, Maryland.

A CHANGE IN THE POPULATION AT RISK

OVER THE PAST SEVERAL DECADES, PROGRESS IN MODERN neonatology and neonatal care on many fronts has made possible the survival of significant numbers of premature infants who have been born at gestational ages and birth weights that previously were thought to be below viability (Table 1[15]; and Matthews T, written communication).

Although the data in Table 1 do not address the issue of ROP directly, they do provide evidence for the magnitude of the growth in survival of infants at birth weights of <750 g and gestational ages of ≤30 WG.

RESULTS OF THERAPY FROM THE RANDOMIZED CLINICAL TRIALS: ZONE 1 VS ZONE 2

THE RESULT FOR THE CLINICIAN WHO EXAMINES AND treats premature infants for ROP is a shift in the presentation of the disease in recent years. ROP is often more posterior in location and more difficult to treat successfully with ablative therapy. In the cryotherapy for retinopathy study, there were 18 treated eyes among 194 eyes (9.3%) that were diagnosed as having zone 1 disease[4]; in the Early Treatment of Retinopathy of Prematurity trial, there were 162 of 674 infants (24%) with zone 1 disease. Table 2 shows the incidence of unfavorable outcome in zone 1 and zone 2 ROP-treated eyes for the three large randomized control clinical trials, cryotherapy ROP,[4] the Supplemental Therapeutic Oxygen in Prevention of ROP[5] trial, and the Early Treatment of Retinopathy of Prematurity trial.[6] The results for the three trials were combined to provide a total incidence of unfavorable outcome for therapy in zone 1 vs zone 2 in the three trials combined. It is clearly evident from these figures that zone 1 ROP is associated with an increased likelihood of unfavorable outcome over zone 2 ROP.

The data presented problems for statistical analysis, that is, the number of patients in the Supplemental Therapeutic Oxygen in Prevention of ROP trial vs the other two trials. Logistic regression identified a significant interaction in the rates of unfavorable outcomes by zone across the

TABLE 2. Zone 1 vs Zone 2 Retinopathy of Prematurity: Outcome of Therapy

Random Trial	Zone 1 Eyes (n)	Unfavorable (%)	Zone 2 Eyes (n)	Unfavorable (%)	Total Treated Eyes (n)
Cryotherapy for ROP[4]	18	77.8	176	26.1	194
Supplemental Therapeutic Oxygen in Prevention of ROP[5]	110	15	333	10	443
Early Treatment of ROP[6]	162	55.2	512	38.8	674
Total	290	42.1	1021	27.5	1311

$P < .001$: odds ratio, 2.2; 95% CI, 1.6, 2.9; ROP = retinopathy of prematurity.

TABLE 3. Odds Ratios and CIs

Trial	Odds Ratio	95% CI	*P* Value
Cryotherapy for Retinopathy	9.1	2.8, 28.9	<.001
Supplemental Therapeutic Oxygen in Prevention of ROP	1.7	0.9, 3.1	.11 (not significant)
Early Treatment of Retinopathy of Prematurity	2.0	1.4, 2.9	<.001

three studies ($P = .035$). To deal with this, odds ratios and confidence intervals were calculated for each of the three studies individually (the data are provided in Table 3).

To summarize the points made in Tables 1, 2, and 3, the era of micropremature survival has arrived and has brought with it this form of zone 1 ROP in increasing numbers. It is this form, zone 1 ROP that is found most commonly in the micropremature infant, that this article addresses. These are the infants that are particularly vulnerable to this most severe form of ROP.

THE INTERNATIONAL CLASSIFICATION OF ROP (ICROP)

THIS CLASSIFICATION HAS SERVED AS THE BACKBONE OF THE randomized trials that were cited earlier and in numerous trials that have been undertaken elsewhere throughout the world.

Figure 1 summarizes the main points of the ICROP classification and describes the acute stage of the disease as seen by the examining physician.[1] It uses three descriptive variables: the location in terms of zones 1, 2, and 3 (zone 1 is innermost, and zone 3 is outermost) extent describes the clock hour positions of the ROP that was observed and stages 1 to 3. The suffix "plus" was affixed to the staging to denote vascular tortuosity and dilation of the vessels that were observed in the posterior pole, which is an ominous prognostic sign.[16,17] Figure 1 details an adaptation of the diagram that was used clinically to illustrate location, extent, and stage variables of the international classification of ROP.

THERAPY IN ZONE 1 VS ZONE 2 ROP RESULTS IN DIFFERENT CLINICAL OUTCOMES

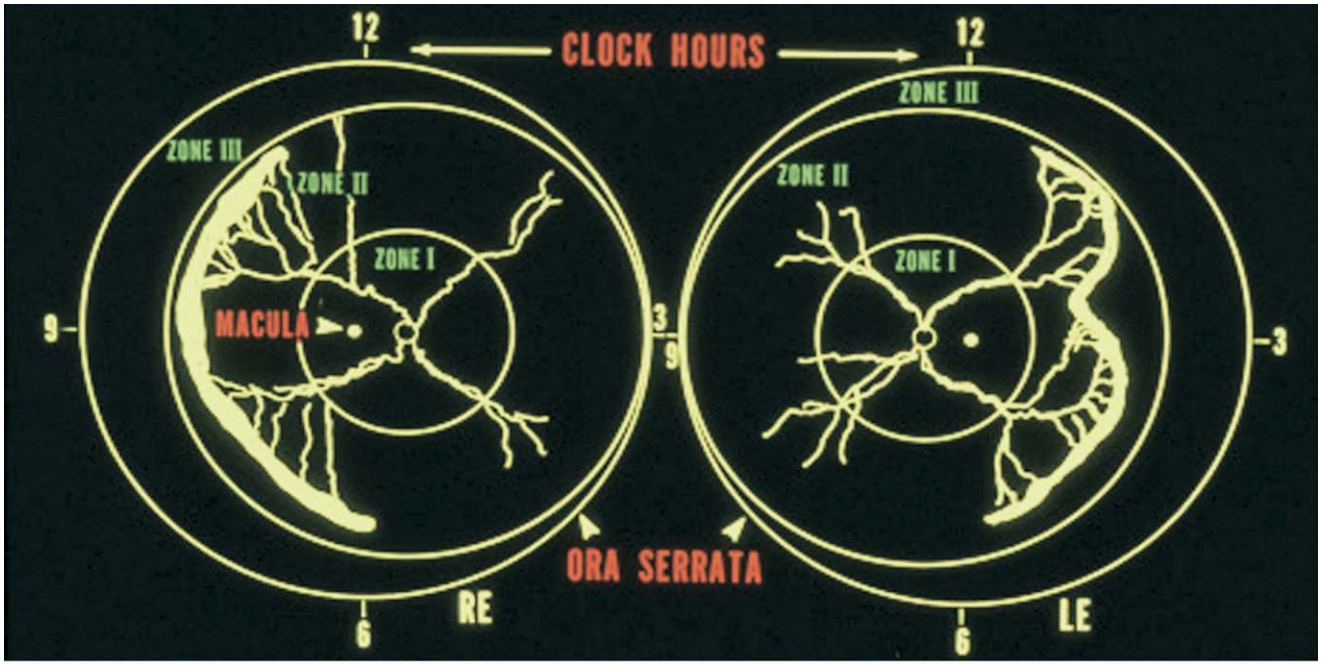
TABLE 2 INDICATES A CLINICALLY AND STATISTICALLY significant difference in the percentage of infants with unfavorable results between the ROP located in zone 1 vs ROP in zone 2. An unfavorable response to therapy is defined (1) a tractional fold of retinal tissue that destroys the normal architecture of the macula or (2) a tractional retinal detachment that involves the macula.[18] Both result in an unfavorable anatomic outcome and an accompanying poor prognosis for normal visual development. Although the definition of exactly what constitutes zone 1 disease has varied somewhat across studies, it always represents the most posterior disease in the eye.

Although both unfavorable responses to therapy in zone 1 and zone 2 ROP result in a poor visual outcome, the differences in the number (percentage) of unfavorable outcomes between the two contiguous zones is striking and heretofore not explained. If it were merely a matter of more unvascularized ischemic retina elaborating more $VEGF_{165}$ signal in zone 1 disease, then ablating larger retinal areas with cryotherapy or laser therapy, which would abolish the source of that signal, should result in comparable outcomes. This is the accepted practice today, yet it has failed to improve the outcome statistic in zone 1 disease.[5]

Our hypothesis is that the difference can be traced to a very specific difference in the response of different blood vessel tissues to injury and reflect the characteristics of the injured tissue, that is, vasculogenic in posterior (zone 1) ROP and angiogenic in more peripheral (zone 2) disease.

CORRELATION BETWEEN ZONE 1 ROP AND THE REGION OF HUMAN RETINAL

- **VASCULATURE FORMED BY VASCULOGENESIS:** Vessels are formed by two distinct mechanisms on differing


12
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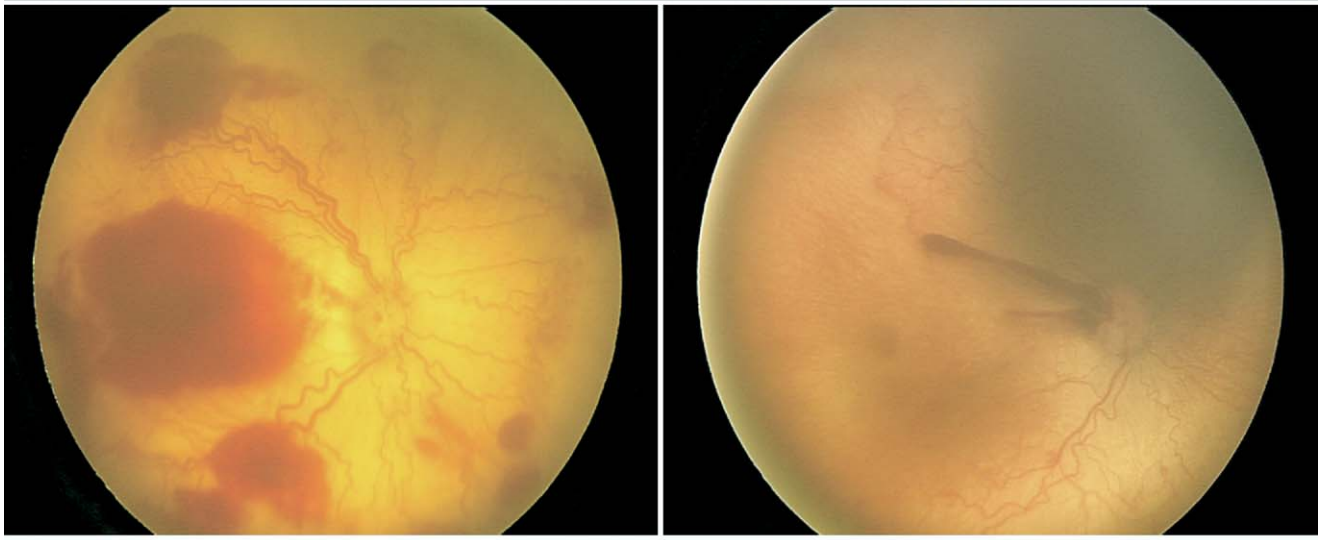


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developmental timetables. We speculate that the distinctions in the location and morphologic condition of ROP disease in different zones suggest that the injured process may be primarily vasculogenic in posterior disease and angiogenic in more peripheral disease. In vasculogenesis, vessels are formed from VPCs that migrate from the optic disk, differentiate, and aggregate to form primitive chords that later develop lumens that are capable of carrying blood cells (Figure 2). The process begins before 14 WG and is complete by 21 WG. The involved cells are ADPase$^+$/Nissl–stained VPCs and are also CD39$^+$ and VEGF receptor–2+.[3,8] CD39 binds to ecto-ADPase and is expressed by human endothelial cells, VPCs, mural cells, and microglia (Chan-Ling T, Baxter LC, Dahl-Strom J, Rosinova E, Bean E and Hughes S: Role of Vasculogenesis and angiogenesis in the formation of human choroids and retina. Association for Research in Vision and Ophthalmology, ARVO Abstracts, 2005). Thus, vasculogenesis is responsible for the formation of the primordial vessel architecture of the central human retina (Figure 2), the major vascular arcades.

Figure 2 shows the outer limits of the region of the human fetal retina that is vascularized at 14 to 15, 18, and 21 WG. The retina at this stage is vascularized by previous invasion of VPCs. By 21 WG, these cells have disappeared. The pattern of vascularization by VPCs is typically "butterfly" in shape and is centered on the optic nerve head. It is clearly noncircular, covers only the central one half of the retina, and shows a four-lobed topography that is indicative of the future artery/vein pairs of superior and inferior retina. This topography closely matches the topography of posterior ROP that has been reported by clinicians. Gallagher and associates[19] have reported the noncircular nature of ROP.

• **ANGIOGENESIS IS MEDIATED BY HYPOXIA-INDUCED VEGF$_{165}$, ALTHOUGH VASCULOGENESIS TAKES PLACE INDEPENDENTLY OF VEGF$_{165}$:** Angiogenesis in which vessels are formed through the budding of endothelial cells from existing vessels completes the process. Angiogenesis is responsible for the formation of the remaining retinal vessels, which includes increasing vascular density (capillary) in the central retina (Figure 3,), completion of all superficial vessels in the peripheral retina (Figure 3, Right), the formation of the deep plexus, and the radial peripapillary capillaries.[3] Figure 4 is a flat mount of a retina at 32 WG. The retina is almost completely vascularized. The primary vascular arcades of posterior retina are evident. Angiogenic sprouting has resulted in a dense capillary network that is evident throughout the retina. Formation of the outer plexus by angiogenesis begins around the incipient fovea between 25 to 26 WG,[3] coincident with the peak period of eyelid opening and the first appearance of the visually evoked potential, which is indicative of a functional visual pathway and photoreceptor activity.[20] The timing and topography of angiogenesis in the human retina supports the "physiologic hypoxia" model of retinal and central nervous system vascular formation.[21,22] Angiogenesis is induced by a transient but physiologic level of hypoxia as a result of the increased metabolic activity of retinal neurons as they differentiate and become metabolically active. This results in the up-regulation of hypoxia-induced factor –1α, which, in turn, induces an up-regulation of VEGF$_{165}$ expression by both astrocytes in the inner retina and Müller cells in the outer retina.[9,23] The new blood vessels bring oxygen and other metabolic requirements, which leads to the down-regulation of VEGF$_{165}$ by neuroglia.

In contrast to the aforementioned mechanism of retinal vessel formation through VEGF$_{165}$ signaling, formation of retinal vessels through vasculogenesis appears to be independent of metabolic demand and hypoxia-induced VEGF$_{165}$ expression.[3,23–25] Evidence for this conclusion includes the observation that (1) substantial vascularization in the human retina occurs before the detection of VEGF$_{165}$ messenger RNA in the retina. At 18 WG, the inner plexus covered approximately 54% of the retinal area; however, VEGF$_{165}$ mRNA was not detected in the human retina by in situ hybridization until 20 WG.[23] (2) Vasculogenesis is well-established by 14 to 15 WG, before the differentiation of most retinal neurons.[23] (3) The topography of the

FIGURE 1. Zones of retinopathy of prematurity (ROP) as defined in the international classification of ROP[1] and digital images of ROP in zone 1. (Top left) The diagram presents the three zones (1, 2, and 3) centered on the optic disk with a schematic outline of ROP located in zone 2, at 5 o'clock extent and probably stage 3 without plus disease. Clinical records of examinations by ophthalmologists often depict the findings of the examination in this fashion. (Top right) Zone 1 ROP demonstrates (a) the 12 o'clock position of circumferential involvement centered on the optic disk; (b) many abnormal blood vessels that lack orientation into temporal and nasal arcades that exit the optic nerve; (c) no clear-cut distinction between the curved orientation of the temporal vessels that embrace the future macula; (d) the vessels themselves end in vascular "tangles"; (e) no wedge-like structures that are typical of posterior zone 2 ROP (see text); and (f) no organized ridge visible to denote the border of vascular and avascular retina. Hemorrhages that are seen in this image often are seen accompanying zone 1 disease because of vascular friability. (Bottom left and right) These color and red-free images illustrate still other aspects of zone 1 ROP vascular involvement: (1) full 12 o'clock involvement but probably later than the Top right image in this Figure, as evidenced by the presence of a formed inferior arcade; (2) a web of vessels that lack organizational structure and end in an edge-like net; (3) the numerous abnormal vessel branches are visible more prominently in the red-free image; (4) as in the Top right portion of the Figure, no ridge at the vascular margin is visible; and (5) hemorrhage or persistence of vessels in the hyaloid canal is a sign of vascular immaturity.

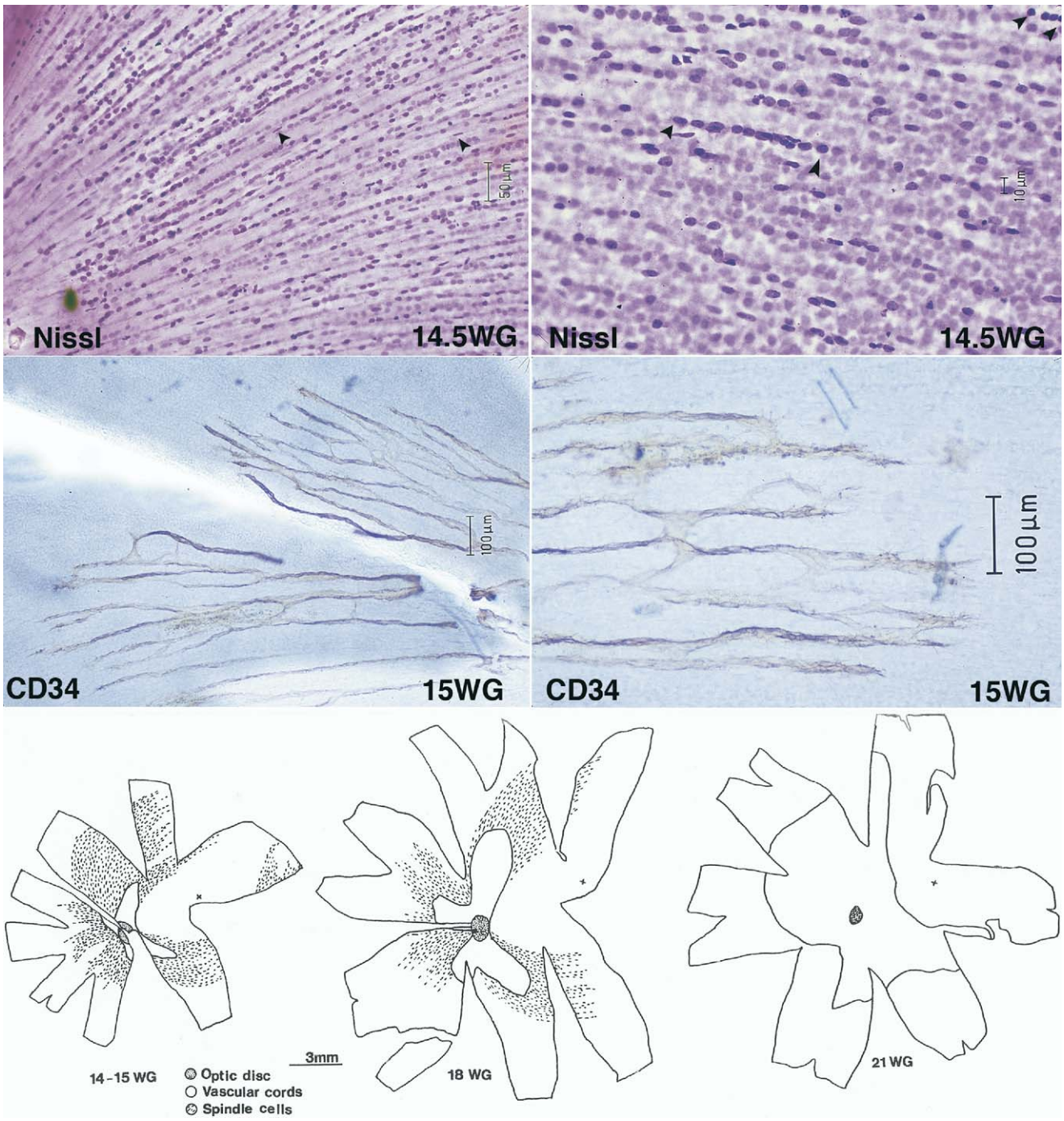

FIGURE 2. Normal development of the human fetal retinal vasculature. (Top left and right) Low- and high-magnification views of a Nissl-stained human retinal whole mount at 14 to 15 weeks of gestational age (WG). Shows a region that is immediately adjacent to the optic nerve head (lower left-hand corner). Large numbers of spindle-shaped cells, which are interspersed among other somas, stream in superficially from the optic nerve head. These spindle-shaped, presumed vascular precursor, cells join to form chords of cells (arrowheads at top right) to form vascular chords. (Middle left and right) Low- and high-magnification view of the first primordial vascular arcades that are labeled with CD34 immunohistochemistry, evident in the region of the optic nerve head at 15 WG. These vascular arcades show the four-lobed topography of formation that is indicative of the future superior and inferior (temporal and nasal) artery vein pairs. Morphologically, they are straight and lack significant capillary density. (Bottom) The distribution of the spindle cells are shown at 14.5, 18, and 21 WG. The stippled regions show the distribution of the spindle cells at each age; the white regions show the areas with vascular cords. With increasing maturity, the outer limit of the vascular cords expanded markedly, whereas that of the vascular precursor cells did not. At 21 WG, no spindle cells were evident in the retina. It is clear from these maps that the area formed by the vasculogenesis is not circular in the developing human retina. The X indicates the location of the incipient fovea.

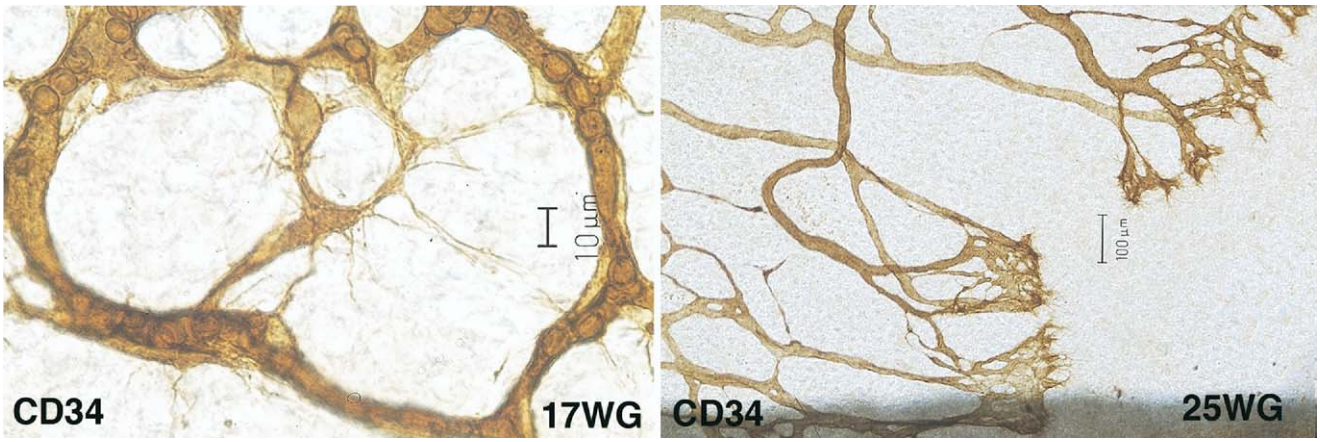

FIGURE 3. In contrast to the vessels formed by the vasculogenesis, increasing capillary density in the central retina and formation of the remaining superficial vascular plexus in the peripheral retina takes place through angiogenic budding. (Left) A high-magnification view of vascular sprouts in a mid peripheral region of the retina at 17 WG. Filopodia extended in numerous directions and then expanded to form vessel segments. (Right) Angiogenic sprouting at the leading edge of vessel formation of a 25-WG infant. (For the full report, see reference 3).

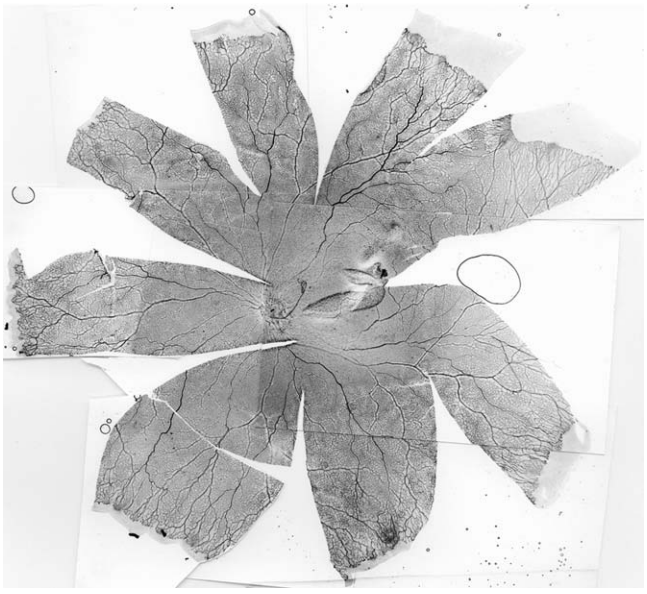

FIGURE 4. A photo montage of a 32-WG human fetal whole mount stained with the use of CD34 immunohistochemistry. The foveal region is wrinkled because of the significant curvature changes at this region. The temporal raphe is shown curving upwards and outwards towards the right. This image displays the developmental aspects of both vasculogenesis in the posterior retinal vessels and angiogenesis in the development of the precapillary and capillary layers between the major vessels (arrow) and in the posterior pole as well.

formation of the primordial vessels by vasculogenesis is centered around the optic disk, whereas neuronal maturation and accompanying vessel formation is centered around the fovea.[25] (4) Further evidence of the independence of vasculogenesis from $VEGF_{165}$ is provided by $VEGF_{165}$ knockout mice. In these animals, in which not only paracrine but also autocrine $VEGF_{165}$ production is lost, vessels still form by vasculogenesis but are highly abnormal.[26] Reduced $VEGF_{165}$ expression in mice that are heterozygous for the $VEGF_{165}$ null mutation is associated with the formation of vessels in the forebrain mesenchyme but not in the neuroepithelium.[26] Given that the formation of vessels in the forebrain mesenchyme is thought to occur by vasculogenesis, whereas this formation within the neuroepithelium is thought to take place through angiogenesis, these observations provide further evidence that vasculogenesis is not dependent on hypoxia-induced $VEGF_{165}$ expression. The positioning of the astrocytes just ahead of the leading edge of vessel formation places them in an ideal position to mediate the angiogenic response to "physiologic hypoxia," through the up-regulation of $VEGF_{165}$ expression.[9]

ZONE 1 ROP: CLINICAL PRESENTATION

FIGURE 1 SHOWS ROP IN THE MOST POSTERIOR ASPECT OF the retina. The vessels in this very posterior location display extreme disorganization. The following features are prominent among the features that they illustrate: (1) in contrast to the normal arcade pattern of distinction between nasal and temporal vessels, numerous tortuous vessels exit the optic disk without any discernable pattern; (2) vessel endings in peculiar "tangles" of smaller vessels (possibly precapillaries?) without any suggestion of the normal orderly dichotomous branching pattern as vessels extend into the periphery; (3) the distinction between artery and vein is blurred, that is, it is difficult clearly to identify artery and vein on color or vessel characteristics; (4) the involvement of this primitive pattern is circumfer-

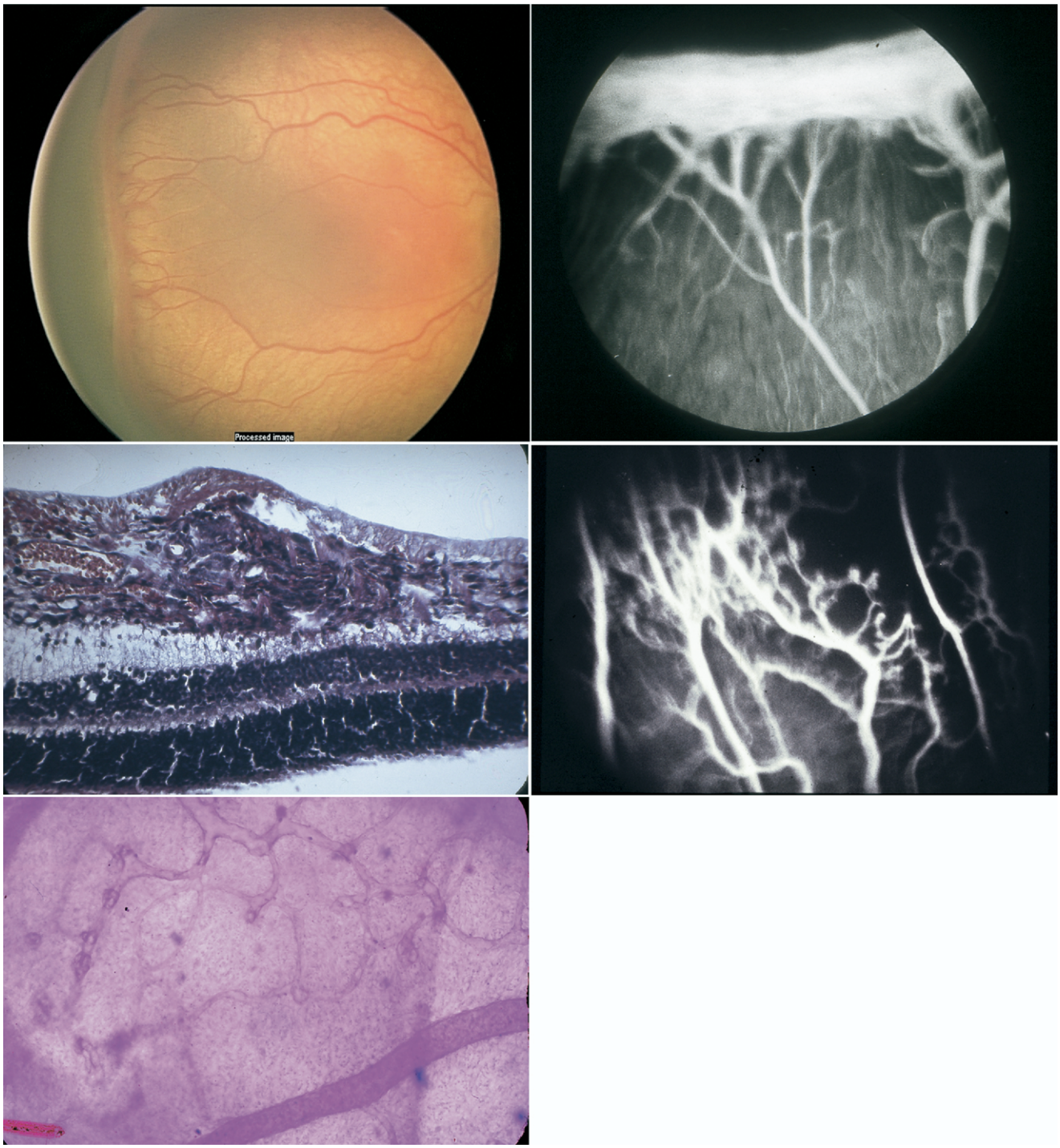

FIGURE 5. Zone 2 retinopathy of prematurity (ROP). These images show the classic picture of zone 2 ROP. (Top left) The color image of the ridge in zone 2 beyond the formed temporal arcades. (Top right) A fluorescein angiogram image of a similar ridge with formed vessels that enter the structure (which leaks the dye profusely in a matter of seconds), absent capillary bed posterior to the structure, and absent vessel formation anterior to the structure. (Middle left) Periodic Acid-Schiff–stained section of the retina (×50) to demonstrate the ridge structure with prominent blood vessels in the nerve fiber layer to the left entering nests of PAS + stained cells with few capillaries within and no evidence of blood vessels beyond (to the right) in the image. (Middle right) Fluorescein angiogram of the retina posterior to ROP ridge in zone 2. Formed capillaries are present in the retina, but they are not normal. Many end in peculiar buds; others appear as anastomotic arterial capillary-to-capillary vessels. (Bottom) Periodic Acid-Schiff–stained whole mount also demonstrates the vessel abnormalities.

ential, centered on the optic disk with no discernable butterfly pattern seen in the early phase of normal vasculogenic organization in the posterior pole; 5) the absence of a ridge-like structure organized at the tips of the vessels, typical of zone 2 ROP; and (6) because of its extreme posterior location, no "wedge" pattern at the vessel tips or between vessels is observed; this wedge is a distinct by-product of the fundamental developmental plan of retinal arcade formation. Although not discernable in these images, but able to be tested with fluorescein angiography, our hypothesis would predict that few or no capillaries would be seen on fluorescein angiography.[27–30] The vessels show no growth into the periphery when observed over time.

In the ROP pictured in zone 1, our hypothesis implicates vasculogenic-, rather than angiogenic-, formed vessels as the injured tissue. The "tangles" are distinctly abnormal structures at the ends of these malformed arteries and veins. They are small in diameter in comparison with the arteries and veins; however, because they are visible to the eye and camera, they are not capillaries. Perhaps they are the structures that would feed into capillary beds and drain them; hence, we surmise that they might be precapillary arterioles and venules. Without further investigation of in vivo and in vitro material, it is not possible to determine an answer at this point.

ZONE 2 ROP: CLINICAL PRESENTATION

FIGURE 5, BY CONTRAST, PRESENT A FAMILIAR PICTURE OF ROP as it was described earlier.[31,32] Illustrative of the differences from zone 1 are (1) the posterior vascular arcades, particularly temporal, that embrace the macula (odd needs fixing) are always present and normally formed. (2) In contrast to zone 1 ROP, a clearly delineated structure identified in ICROP[1] as a ridge stage 2 or a ridge with extraretinal vascularization; stage 3 is always present at the tips of the vessels. This structure, we suggest, is typical of arrested angiogenesis. In zone 2 ROP, we observe clinically, as the earliest sign of ROP, a thin white line in the retina at right angles to the tips of developed vessels (demarcation line: stage 1). Over time, that line thickens in place and develops a third dimension (ridge: stage 2). The structure fills with fluorescein dye and leaks the dye profusely (Figure 5). During that interval, it does not move in the retina and is contained within the internal limiting membrane (Figure 5). Vessels (arteries and veins) are seen entering and draining the structure. We submit that this occurs only during angiogenesis. This clinical picture is familiar to every clinician who has studied the evolution of ROP since it was first described.[31,32] (3) In very posterior angiogenic disease, the ridge structure is indented to form a "wedge" where the "butterfly wings," that is, the branches of major vessels that feed the ridge approach one another. (4) Vessel morphologic condition clearly allows the differentiation of artery and vein, although over time both become distorted by dilation and tortuosity to form "plus" disease.[16,17] (5) Fluorescein angiography demonstrated the presence of capillary formation (Figure 5), although not a normal capillary bed in the vascularized posterior retina.[28–34] (6) The shunt structure can be discontinuous; although this is not illustrated in these images, it is observed more clearly in more peripheral zone 2 disease.

THE WEDGE IN ZONE 2 ROP: IMPLICATIONS FOR THE OVER DIAGNOSIS OF ZONE 1 ROP

THE WEDGE IN ZONE 2 ROP (FIGURE 6) HAS ITS BASIS IN THE natural topography of human retinal vascularization.[3] The "wedge" arises naturally from the way the vasculogenesis proceeds from the optic disk. As it spreads out from the disk, it assumes four ellipses in shape (two temporal and two nasal) that resemble a butterfly-wing distribution. Vessel growth in the nerve fiber layer proceeds where the ellipses lose their natural overlap, and a wedge develops between them.

At this point, it is important to note a decision that is reached by the clinicians who plan the original cryotherapy ROP randomized trial.[4,18] In 1985, the group was struggling to define the zonal interpretation of ROP in terms that provided a guideline of the severity of the disease for clinicians. They made an educated guess. Unfortunately, it was an incorrect one. It was believed that any ROP that extended into zone 1, even if the rest of the disease were clearly in zone 2, classified that eye as a zone 1 ROP eye. The wedge naturally appeared to do just that. The wedge thus became a hallmark of zone 1 disease for that study and others studies to follow. We believe that decision has lead to the over-classification of eyes as zone 1 eyes when, in fact, the vascular anatomy of zone 1 was intact and complete. The misclassified disease was angiogenic and truly $VEGF_{165}$-modulated zone 2 disease. Why is that important? Because it led to an inflation of the number of zone 1 eyes that were classified as successful for ablative therapy and consequently lessened the number of therapeutic failures of ablative therapy for these eyes. Put another way, over-calling the number of successful cryo- or laser therapies as zone 1 ROP eyes when, in fact, they had the specific hallmarks of posterior zone 2 (angiogenic) eyes (posterior arcades complete, a ridge typical of angiogenesis, and capillary formation (a product of the budding process) well advanced) led to exaggerated successes in the cryo- or laser therapy of ROP. Success is not unexpected in zone 2 eyes, which is what they truly were. In summary, we suggest that the wedge is a natural consequence of the completion of the anatomic arcades that are formed by vasculogenesis that is complete or near complete at this

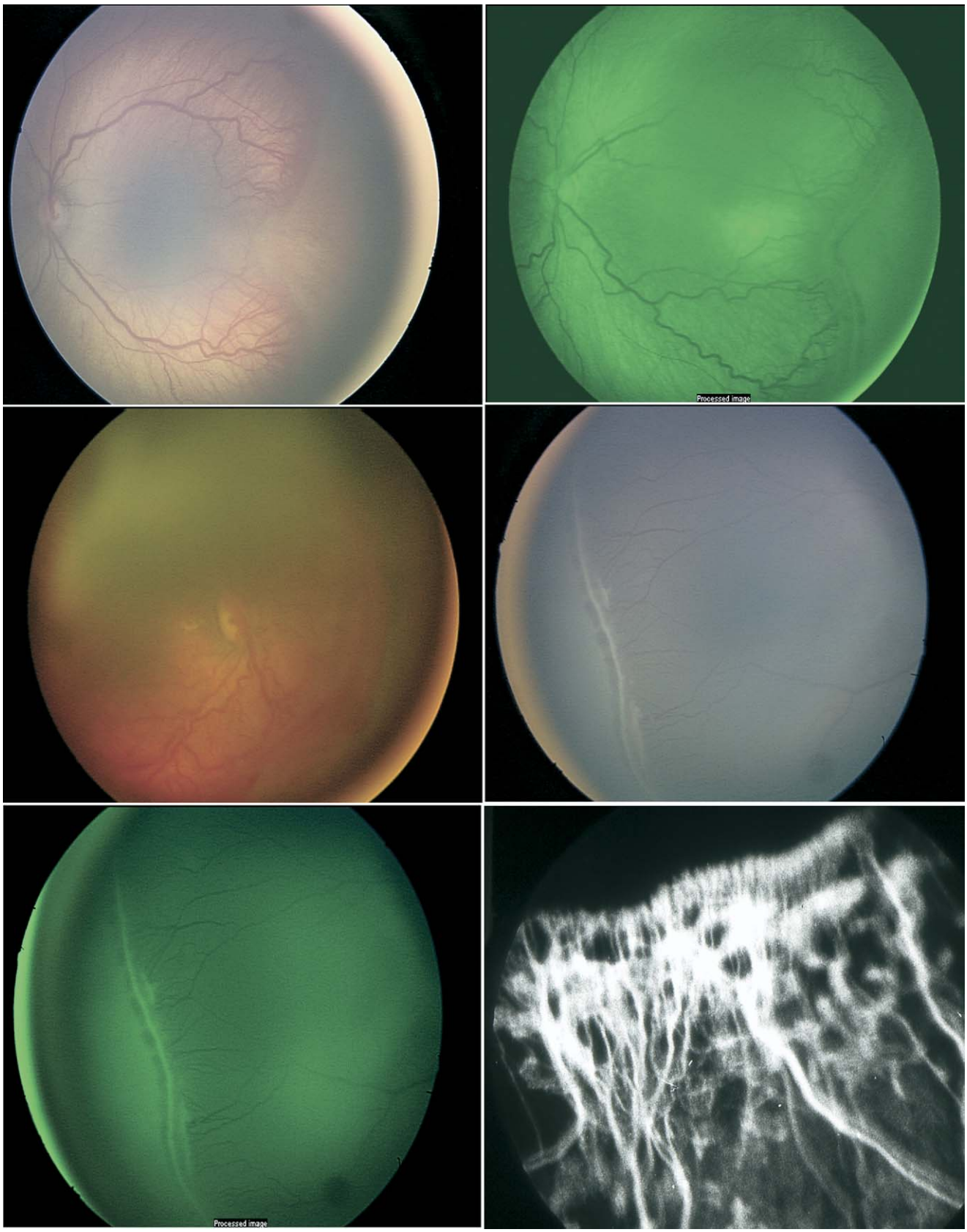

FIGURE 6. Characteristics of the wedge underlying the misclassification of zone 2 retinopathy of prematurity (ROP) as zone 1 ROP; hybrid form of ROP, early regression, color and red-free; fluorescein angiogram of early regression. (Top left) Color digital image of the "wedge" that is seen in the very posterior zone 2. Note the intact formation of the temporal arcades embracing the

TABLE 4. Clinical Presentation of Zone 1 vs Zone 2 Retinopathy of Prematurity

Zone 1 ROP (Vasculogenic)	ROP Characteristic	Zone 2 ROP (Angiogenic)
Posterior pole	Location	Outside posterior pole
Always 12 o'clock hours	Extent	Variable extent
Normal arcades absent	Vascular pattern	Normal arcades preserved
Absent	Shunt	Present
Never present	"Wedge"	Often present
Precapillary tangles	Microvascular pattern	Layered capillaries
Never?	Regression	Common
Poor (55.2% unfavorable[6])	Response to therapy	50% reduction in unfavorable outcome[4]

ROP = retinopathy of prematurity.

stage. The wedge pattern is, in fact, the most posterior form of a zone 2 ROP lesion.

HYBRID FORM OF ROP

IT IS NOT UNEXPECTED IN A DEVELOPING BIOLOGIC SYSTEM that there would occur a certain amount of overlap between vasculogenesis and angiogenesis in the course of development (Figure 6). The upper temporal arcade (unfortunately not visible in Figure 6, but visible on examination) is formed normally; the vessels formed in place of the lower arcade are distinctly abnormal. Our interpretation of this formation of the vessels that exit the optic disk is that both the vasculogenic and angiogenic processes of vascular formation at the very posterior aspect of the retina have been interfered with. The massive hemorrhage overlying the tissues in the inferior aspect of the image is a common occurrence, particularly in vessel fields where florid, albeit abnormal, development produces large masses of abnormal primitive vascular tissue. These tend to bleed easily.

SPONTANEOUS REMISSION OF ROP

THIS FASCINATING PHENOMENON, REMISSION OF ROP that occurs without intervention of any kind, has never been explained satisfactorily. Our reanalysis of the pathogenesis of zone 1 vs zone 2 ROP in terms of vasculogenesis and angiogenesis can now, at least partially, explain this phenomenon. It occurs most often, but not exclusively, in mild ROP, which is peripheral in location. Characteristic of the process is that it always begins with the budding of capillaries invading avascular retina. The earliest clue to its development is the occurrence of small loops of translucent retina that are anterior to the ridge (Figure 6). These loops are areas in which capillaries that are growing from the anterior "vanguard" (as it was named by Ashton[35]) invade the avascular retina and, thereby, result in perfusion by red blood cells and nutrient exchange at the capillary-tissue interface. A fluorescein angiogram of the process (Figure 6) shows the presence of these competent capillaries that are filled with and retain the dye as evidence of their competence, growing into previously avascular retina.

IMPLICATIONS OF THIS STUDY

WE HAVE PRESENTED EVIDENCE THAT ROP MAY BE THE result of two distinct clinical-pathologic processes, both of which are capable of devastating damage to the developing eye. They resemble each other in certain respects but differ substantially in their location in the retina, the clinical appearance of their fundamental lesions, and their response to therapy. Indeed, therapeutic successes in zone 1 disease, in fact, may be because of the misclassification as zone 1 ROP disease that rightfully belongs to be classified as zone 2 disease. Table 4 summarizes the differences

macula region posterior to the structure. (Top right) Red-free image to enhance the visible vascularity of the structure. Visible on the lower anterior arc of the wedge are several darkened areas that are suggestive of the early regression of the ROP in that region. (Middle left) Digital image of a hybrid form of zone 1 to 2 ROP. The upper temporal arcade, although poorly visualized in this image, appeared normal. The vessels in the lower arcade are abnormal, resembling the abnormal vessels of zone 1 ROP and ending in a mass of friable tissue that lacks clearly defined vessels. (Middle right) The earliest clinical sign of regressions: Transparent arcs of retinal tissue that appear as darkened loops anterior to the ridge. This signifies competent capillary growth into the previously avascular retina. (Bottom left) Red-free image of the same area. (Bottom right) Fluorescein angiogram of regression with a border of capillary growth at the anterior edge of the ridge that signals the process of involution of the ridge structure and vascularization of the previously avascular retina.

between zone 1 and zone 2 ROP, as observed with indirect ophthalmoscopy.

We have provided both anatomic and clinical evidence in support of our hypothesis that the different stages that are observed in ROP can be attributed to two distinct pathologic pathways of disease. Our hypothesis is that zone 1 ROP takes place in the posterior retina, which is where vascularization occurs through vasculogenesis. Zone 2 (and zone 3) ROP takes place over areas of the retina that are vascularized through angiogenesis. Our studies of normal human fetal retinal vascularization have shown that vasculogenesis, which involves previous invasion of VPC cells, is responsible for the formation of the primordial vessels that are seen at the central posterior region of the human retina, whereas angiogenesis formation through budding from existing blood vessels are responsible for the formation of the remaining retinal blood vessels in the human retina. As a consequence, the course of ROP in these locations also follows distinct cellular and molecular cues.

In support of this conclusion, we have shown that zone 1 ROP is clinically less responsive to cryo- and laser therapy than is zone 2 ROP. According to the physiologic hypoxia model of retinal vascularization,[21,22] the reason that ablative therapy is effective in the treatment of ROP is the fact that such ablation by both modalities results in the destruction of the neurones and perhaps other sources of $VEGF_{165}$ in the avascular retina that includes that retina in the immediate vicinity of the disease, thus taking away the hypoxia-induced stimulus to produce $VEGF_{165}$. Our observation that zone 1 ROP is less responsive to laser therapy supports the conclusion that zone 1 ROP may be driven by some other molecular signal than $VEGF_{165}$ alone. An alternative interpretation of this could be that a second source of $VEGF_{165}$, such as vitreal macrophages as reported by Naug and associates,[36] is responsible for the lack of effect of laser therapy in zone 1 disease.

The arbitrary clinical decision to include a wedge of ROP involvement as little as 1 o'clock hour position as zone 1 disease should be dealt with here. This wedge is most typically, but not exclusively, seen on the temporal retina at or near the horizontal raphe. Its apex pointing to the optic disk is the evidence that is taken often to imply that this is the "1 o'clock hour" necessary to call this zone 1 disease. This wedge, we assert, arises naturally from the geometry of normally formed vascular ellipses as they move away from each other, thereby losing overlap and creating the wedge structure. The point in time at which this occurs is at or near the end of the vasculogenic era. At that point, if ROP is present and involves the wedge, it is angiogenic in nature and manifestly a zone 2 eye.

A further implication of this work is that it provides a firmer, more rational basis for the forms of ROP that are encountered by the clinician. A classification that is based on the known developmental anatomy of retinal vasculogenesis is at the heart of the work that is presented here. The international classification of ROP served an extraordinary need at the time of its development in that it united all the observations that were made by clinicians of the disease as they encountered it. In so doing, it provided a common language that could be shared by clinicians worldwide. The studies that resulted from it demonstrated its usefulness. It was not based, however, on any firm knowledge of how retinal vessels were formed nor on any knowledge of the processes that directed their formation. We believe the work presented here provides such a framework for the conception and development of a classification, incorporating these ideas without sacrifice of the essential elements of ICROP. The recent publication by a group of clinicians, many of whom played leading roles in the original development of ICROP, is a step in the right direction.[37]

As was pointed out by Kivlin and associates[38] in their study of infant eyes, cryotherapy ROP in which the retinal vessels are located by the examiner has prognostic significance for the outcome of ROP. In their study, the chance of developing threshold ROP was related inversely to the early degree of retinal vessel development, as was the chance of an unfavorable anatomic and visual outcome. As they stated in their paper, vessel development was an independently important factor, even when birth weight, gestational age, and race were considered. Also as pointed out in their paper, 58% of eyes with zone 1 disease had a total retinal detachment as their outcome at 3.5 years.

Because of the insensitivity of vasculogenic precursor tissue to $VEGF_{165}$ modulation and as a consequence of frequent failure of current ablative therapy, other approaches to therapy must be considered. On an experimental basis, studies that are directed at the inhibition of pathologic vasculogenesis (zone 1 ROP) might take a direction other than ablative therapy. For example, with the use of agents such as bevacizumab[39,40] or imatinabmesylate,[41–43] monoclonal antibodies may inhibit tyrosine kinase proteins and attack the process of abnormal vasculogenesis upstream from $VEGF_{165}$.

Another implication of this work is that it raises, but does not settle definitively, the possibility that part of the pathologic changes that result in ROP may occur before the age of survivability. This is not a novel idea, because recent work on the cause of cerebral palsy has developed evidence that cerebral palsy is due to early brain developmental injury and not to traumatic birth.[44]

Michaelson[45] and Cogan[46] in the 1940s and 1950s described both an angiogenic and a vasculogenic mechanism of forming retinal blood vessels. Michaelson clearly described the budding of vessels from already formed vessels in the kitten, and Cogan described vessel formation arising de novo from a mesenchymal precursor cell. The controversy was never resolved satisfactorily. It turns out that both men, who were giants of their age in scientific research and clinical ophthalmology, were right.[46]

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Biosketch

John T. Flynn, MD, is a Pediatric Ophthalmologist with a life-long interest in Retinopathy of Prematurity. His early years were spent at Bascom Palmer Eye Institute in Miami, Florida. Dr Flynn retired from BPEI in 2000 and joined the faculty at the Harkness Eye Institute of Columbia University where he currently occupies the Ann Cohen Chair of Ophthalmology. Dr Flynn major interests are teaching, patient care, and research in the blinding eye diseases of children.

Biosketch

Tailoi Chan-Ling, PhD, has a record of contribution to the understanding of the development of the mammalian retina, in particular the glial, neuronal, and vascular interactions during formation of the retinal vasculature. Studies in the human fetal retina have shown the role played by both vasculogenesis and angiogenesis in the formation of the human retinal vasculature. Dr Chan-Ling studies using the kitten model of Retinopathy of Prematurity (ROP) have shown the role of astrocyte degeneration and vascular endothelial growth factor (VEGF) in the pathogenesis of ROP, and the ability to minimize pathology and control the rate of retinal re-vascularization using supplemental oxygen therapy.